UNIVERSITÉ DU QUÉBEC À MONTRÉAL

ACCLIMATATION HIVERNALE CHEZ UN PETIT ENDOTHERME : LA MÉSANGE À TÊTE NOIRE (*POECILE ATRICAPILLUS*)

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MAGALI PETIT

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«Savoir pour prévoir, afin de pouvoir»

Auguste Comte

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BMR	Basal metabolic rate
CMR	Capture marquage recapture
DEE	Daily energetic expenditure
FER	Forêt d'enseignement et de recherche
GLM	General linear model
HCT	Hématocrite
LCT	Lower critical temperature
LME	Linear mixed effect
LRT	Likelihood ratio test
M_b	Body mass
MMR	Maximal metabolic rate
Msum	Summit metabolic rate
T_b	Body temperature
TNZ	Thermoneutral zone

RÉSUMÉ

Les changements globaux sont associés à une augmentation de la stochasticité de l'environnement pouvant affecter la phénologie, la démographie et la variabilité génétique des espèces. La flexibilité phénotypique permet aux organismes d'ajuster leur phénotype à court terme, ce qui devrait leur conférer une certaine capacité à affronter les fluctuations environnementales. Dans ce contexte, il est donc important d'étudier la capacité de réponse des individus aux variations de leur environnement afin de comprendre l'effet des changements environnementaux sur la dynamique des populations. Cependant, nous manquons d'étude en conditions naturelles sur la capacité d'ajustements à court terme de paramètres physiologiques chez les endothermes. Avec cette thèse de doctorat, nous avons utilisé une population de mésanges à tête noire (*Poecile atricapillus*) résidant au Québec pour étudier les ajustements hivernaux du métabolisme de base (BMR) et de la capacité thermogénique maximale (Msum) en conditions naturelles. Plus particulièrement, les objectifs de cette thèse étaient de mettre en évidence 1) les patrons d'ajustements du BMR et du Msum (***chapitre 1***), 2) la relation entre les conditions météorologiques et la performance métabolique (***chapitre 2***), 3) l'effet de la composition corporelle sur le BMR et le Msum (***chapitres 3 et 4***) et 4) le lien entre le phénotype hivernal et la valeur sélective (***chapitre 5***).

Avec le ***chapitre 1***, nous avons observé, pour la première fois en milieu naturel, qu'un oiseau de petite taille est capable d'ajuster son métabolisme hivernal à court terme. Nos résultats ont montré une augmentation hivernale du BMR (6%) et du Msum (34%) et ont révélé un découplage temporel entre ces deux paramètres. Alors que l'augmentation du BMR ne commençait qu'en novembre pour revenir à un niveau estival dès mars, le Msum commençait à augmenter dès la fin de l'été et restait élevé en mars. Ce décalage temporel entre BMR et Msum suggère que les deux paramètres métaboliques répondent à des contraintes hivernales différentes.

Avec le ***chapitre 2***, nous avons décrit, pour la première fois chez une espèce aviaire en liberté, les normes de réaction du BMR et du Msum en fonction du gradient naturel des conditions météorologiques. Les résultats ont révélé que le métabolisme de base différait entre les individus et était faiblement et négativement relié à la température minimale. Quant à la capacité thermogénique, nos données ont montré que les mésanges ajustaient leur Msum avec la température minimale selon une courbe sigmoïde, augmentant linéairement entre 24°C et -10°C, et avec l'humidité absolue selon une courbe en U. Ces résultats impliquent que les coûts de maintenance seraient surtout individu-dépendants et ne seraient que faiblement influencés par les conditions météorologiques alors que la capacité thermogénique serait, au contraire, principalement liée aux conditions météorologiques, notamment la température. Avec le ***chapitre 3***, en manipulant la taille des muscles par un protocole de réduction de surface alaire, nous avons démontré, pour la première fois en milieu naturel, le lien de cause à effet entre la taille des muscles pectoraux et la capacité thermogénique maximale. Nos résultats ont aussi montré que le Msum était positivement corrélé à l'hématocrite. Cette étude démontre que chez un oiseau de petite taille en conditions naturelles, la thermogénèse par frissonnement est supportée par les muscles pectoraux et probablement par la capacité de transport de l'oxygène.

Le ***chapitre 4*** nous a permis d'étudier le lien entre la variation de masse des tissus et les ajustements du BMR et du Msum au cours de l'année. Nos résultats ont montré que 64% de la

variation annuelle du Msum était expliquée par les changements de masse des muscles et des organes cardio-pulmonaires alors que 35% de la variabilité annuelle du BMR était liée à la masse des muscles squelettiques et des organes excréteurs (foie+reins). Finalement, les résultats du ***chapitre 5*** ont montré que, chez un petit passereau résident, la survie intra-hivernale était liée au Msum suivant une courbe sigmoïde alors que la survie à long terme était indépendante du phénotype hivernal. Cette étude met en évidence, pour la première fois chez une population naturelle d'oiseaux, une relation positive entre le métabolisme hivernal et la valeur sélective.

Mots-clefs : flexibilité phénotypique ; endotherme ; acclimatation hivernale ; performance métabolique ; oiseau ; métabolisme de base ; capacité thermogénique maximale.

ABSTRACT

Global changes are associated with an increase in weather stochasticity, which could affect phenology, demography and genetic variability of species. Phenotypic flexibility, which allows individuals to adjust their physiology rapidly to variations of their habitat should provide a certain capacity to buffer environmental fluctuations. In this context, it is therefore crucial to study response capacity of individuals to surrounding variations in order to understand the effect of environmental changes on population dynamics. However, as far as we know, there is a lack of study on capacity for short-term adjustments of physiological parameters in free-living endotherms. With this thesis, we used a population of Black-capped chickadees (*Poecile atricapillus*) living in Quebec to study winter adjustments of basal metabolic rate (BMR) and maximal thermogenic capacity (Msum) in natural conditions. More specifically, the objectives of this thesis were to highlight 1) the intra-seasonal pattern of BMR and Msum adjustments (***chapter 1***), 2) the relationship between weather conditions and metabolic performance (***chapter 2***), 3) the effect of body composition on BMR and Msum (***chapters 3 and 4***) and 4) the link between winter phenotype and fitness (***chapter 5***).

With ***chapter 1***, we showed for the first time in natural conditions that this small avian species is able to adjust its winter metabolism on a short time scale. Results revealed a winter increase in BMR (6%) and Msum (34%) and a temporal mismatch between these parameters. While BMR began its seasonal increase in November and came back at a summer-like value as soon as March, Msum began to increase at the end of summer and stayed high in March. This difference in the timing of variation in winter BMR and Msum suggests that the two parameters respond to different winter constraints. With ***chapter 2***, we described, for the first time in a free-living bird species, reaction norms of BMR and Msum across the natural range of weather variations. Results showed that BMR varied between individuals and was weakly and negatively related to minimal temperature. Data revealed that Black-capped chickadees adjusted their Msum with minimal temperature following a sigmoid curve, increasing linearly between 24°C and -10°C, and with absolute humidity following a U-shape relationship. These results suggest that weather conditions have minimal effects on maintenance costs, which would mainly be individual-dependent. In contrast, thermogenic capacity would mainly respond to weather conditions, especially temperature. With ***chapter 3***, using a feather clipping protocol to manipulate muscle size, we demonstrated, for the first time in natural conditions, the causal effect of pectoral muscle size on maximal thermogenic capacity. Results also highlighted that Msum is positively correlated with hæmatocrit. These findings therefore demonstrate that in a small-bodied free-living bird, shivering thermogenesis is supported by pectoral muscle size and probably also by oxygen carrying capacity. In ***Chapter 4***, we studied the relationship between changes in body composition and metabolic performance over the year. Results showed that 64% of Msum variations throughout the year were explained by changes in body muscles and cardiopulmonary organs while 35% of the annual BMR variability was related to variations in mass of body muscles and excretory organs (liver+kidney). Finally, results of ***chapter 5*** showed that, in a small resident passerine, within winter survival was related to Msum following a sigmoid relationship while long-term survival was not dependent on winter phenotype. This study

revealed, for the first time in free-living birds, a positive relationship between winter thermogenic capacity and fitness.

Keywords: phenotypic flexibility; endotherm; winter acclimatization; metabolic performance; bird; basal metabolic rate; maximal thermogenic capacity.

INTRODUCTION GÉNÉRALE

I.1 Modifications de l'environnement et ajustements phénotypiques

Outre un réchauffement global et une élévation du niveau des océans, les changements climatiques s'accompagnent d'événements météorologiques extrêmes (*e.g.* sécheresse, pluie verglaçante) de plus en plus fréquents (IPCC, 2013). Les populations animales dont les individus expriment une capacité de réponse phénotypique (*i.e.* physiologique, comportementale) insuffisante pour faire face à l'augmentation de stochasticité de l'environnement pourraient ainsi être défavorisées ou amenées à disparaître (Canale & Henry, 2010; Forcada *et al.*, 2008). Pour faire face aux variations environnementales, les organismes peuvent s'ajuster à trois échelles de temps (Seebacher & Franklin, 2012) (figure I.1). À l'échelle des générations, l'adaptation génétique permet aux populations de s'adapter aux changements de leur habitat (Hoffmann & Sgrò, 2011). Au cours de la croissance, la plasticité phénotypique permet à un individu juvénile de développer un phénotype adulte adéquat à son milieu (Travis *et al.*, 1999). Durant la vie adulte, la flexibilité phénotypique permet à un individu d'ajuster son phénotype de façon rapide et réversible aux variations de son environnement (Piersma & Drent, 2003). Par conséquent, le maintien des populations dans leur aire de répartition actuelle dépend de leur capacité à répondre non seulement aux changements climatiques à long terme, *via* des mécanismes d'adaptation, mais aussi aux changements à court terme, *via* la flexibilité phénotypique. Les individus ayant une capacité d'ajustement insuffisante pour suivre la vitesse des fluctuations environnementales n'auraient d'autres choix que de se déplacer vers d'autres habitats pour survivre (Canale & Henry, 2010; Reed *et al.*, 2010; Van De Pol *et al.*, 2010). Il est donc essentiel d'étudier la capacité d'ajustement des organismes pour comprendre l'effet de la variation de l'environnement sur leur valeur sélective, sur la dynamique des populations et sur le devenir des espèces.

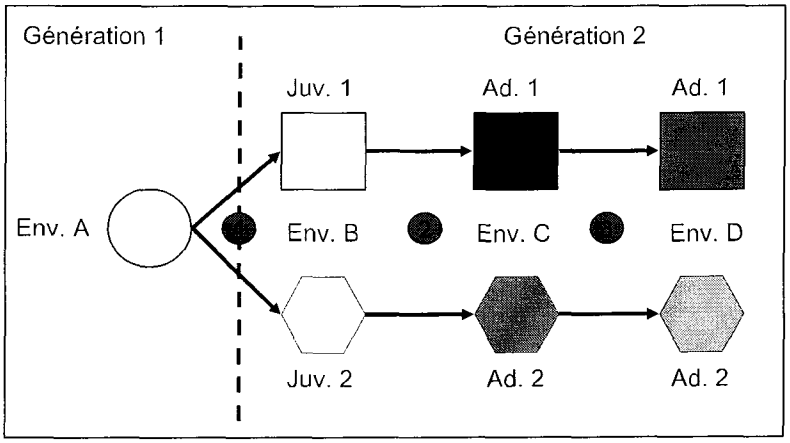

Figure I.1 Réponses compensatoires. Trois processus permettent aux individus de s'ajuster à leur environnement (Env.): 1) l'adaptation génétique entre générations, 2) la plasticité phénotypique entre les stades juvénile (Juv.) et adulte (Ad.) et 3) la flexibilité phénotypique chez les adultes en réponse aux variations environnementales. Différentes formes représentent différents individus et différentes couleurs représentent différents phénotypes.

I.2 Budget énergétique et allocation des ressources.

La valeur sélective d'un individu dépend principalement de sa capacité à acquérir chaque jour suffisamment d'énergie pour assurer ses activités (*e.g.* thermorégulation, reproduction) (Brodin, 2007) (figure I.2). Les ressources énergétiques proviennent de l'alimentation et dépendent de la qualité de la nourriture (*e.g.* contenu lipidique), de la capacité de l'animal à acquérir et ingérer cette nourriture ainsi que de sa capacité à en assimiler l'énergie. Les dépenses énergétiques quotidiennes, quant à elles, (DEE pour « daily energy expenditure ») englobent l'ensemble des activités réalisées par un individu (*e.g.* locomotion, digestion, reproduction, thermorégulation) (Piersma, 2002; Ricklefs *et al.*, 1996).

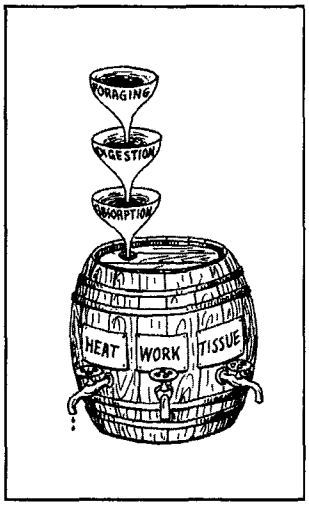

Figure I.2 Représentation du budget énergétique selon le modèle du baril. Après digestion et absorption, l'assimilation des nutriments apporte l'énergie nécessaire à l'organisme (le baril) pour répondre aux dépenses énergétiques (les robinets) associées au travail musculaire, à la croissance des tissus et à la production de chaleur. D'après Weiner (1992).

La somme des apports et des dépenses énergétiques constitue le budget énergétique. Plus un budget énergétique est positif, plus les individus peuvent être performants puisqu'ils ont plus d'énergie à allouer à leurs activités. Par contre, dans un environnement contraignant, où les besoins énergétiques sont accrus et les ressources limitées, des individus peuvent avoir un budget énergétique négatif et donc trop peu d'énergie disponible pour maintenir toutes leurs activités (*e.g.* défenses immunitaires), ce qui à long terme pourrait compromettre leur valeur sélective. Selon le principe d'allocation des ressources (Daan *et al.*, 1990; Weathers & Sullivan, 1993; Weiner, 1992), dans une situation énergétiquement difficile, les individus utilisent des ajustements phénotypiques afin de diminuer leurs dépenses et/ou d'augmenter leurs apports et rééquilibrer leur budget énergétique (Masman *et al.*, 1986).

I.3 L'hiver sous les hautes latitudes, une saison contraignante

L'hiver en régions tempérées froides est considéré comme un défi énergétique majeur pour les espèces homéothermes résidentes (Cooper, 2000; Weathers & Sullivan, 1993). Les froides températures obligent les espèces qui restent actives à augmenter les dépenses énergétiques

destinées à leur thermorégulation afin de maintenir leur température corporelle stable (Cooper & Swanson, 1994; Liknes & Swanson, 1996) alors que la courte durée du jour et la couverture neigeuse entravent leur quête alimentaire et donc potentiellement leurs apports énergétiques (McNamara *et al.*, 1990; Swanson, 2010). Pour survivre à ces conditions hivernales exigeantes, les espèces résidentes ajustent leur phénotype, c'est l'acclimatation hivernale. Ce processus, composé d'un ensemble d'ajustements physiologiques et comportementaux, permet d'augmenter les dépenses énergétiques destinées à la thermorégulation en dépit d'un accès limité à la nourriture (Cooper & Swanson, 1994; Swanson, 1991a; Swanson & Thomas, 2007).

I.4 La thermorégulation

I.4.1 La zone de thermoneutralité

La zone de thermoneutralité (TNZ pour « thermoneutral zone »), délimitée par les températures critiques inférieure (LCT pour « lower critical temperature ») et supérieure (UCT pour « upper critcal temperature ») (figure I.3), correspond à la plage de températures ambiantes dans laquelle un animal endotherme n'a besoin de fournir aucun travail métabolique pour maintenir sa température corporelle constante (Hill *et al.*, 2004). C'est dans cette plage de températures que le métabolisme de base (BMR pour «basal metabolic rate ») peut être mesuré. Le BMR se mesure chez un individu adulte, à jeun, à thermoneutralité et durant la phase inactive du cycle circadien. Le métabolisme de base représente donc l'énergie minimale nécessaire à un individu endotherme pour assurer sa maintenance physiologique (*e.g.* battements cardiaques, filtration rénale) sans prendre en compte les dépenses énergétiques associées au développement, à la digestion, à la thermorégulation et aux activités journalières (Daan *et al.*, 1990; McKechnie, 2008).

Un animal exposé à une température hors de sa TNZ doit par contre ajuster sa physiologie et/ou son comportement (Hayes & Garland, 1995; Seebacher, 2009) pour maintenir sa température corporelle stable. Ces ajustements engendrent une augmentation des dépenses énergétiques et ainsi une augmentation du métabolisme au-dessus du BMR (figure I.3) (Bush *et al.*, 2008; Cooper & Swanson, 1994; Piersma *et al.*, 1995).

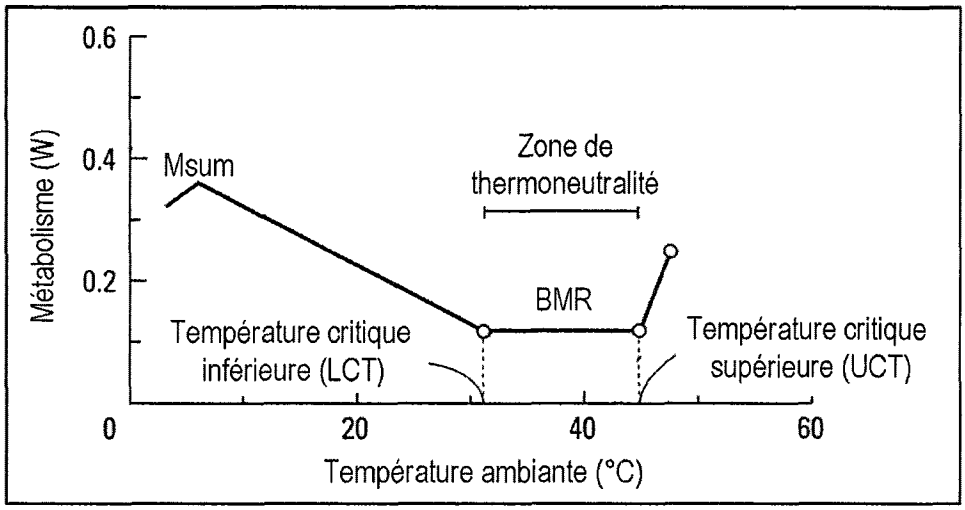

Figure I.3 Relation entre la température ambiante et le métabolisme d'un animal endotherme. Dans la zone de thermoneutralité, un individu adulte, au repos et à jeun exprime son métabolisme de base (BMR). Hors de la TNZ, le métabolisme augmente. Quand la température ambiante devient trop froide, l'animal atteint sa capacité thermogénique maximale mesurable *via* son métabolisme maximal, le Msum. A ce stade, l'individu entre en hypothermie et son métabolisme diminue. Modifié d'après (Willmer *et al.*, 2005).

I.4.2 Les réponses comportementales aux froides températures

Les échanges de chaleur entre un animal et son environnement se font a) par conduction, directement entre deux matériaux en contact (Willmer *et al.*, 2005); b) par convection *via* un fluide (air ou eau) (Hill *et al.*, 2004); c) par radiation thermique *via* l'émission d'ondes infrarouges (Hill *et al.*, 2004) et d) par évapotranspiration (Randall *et al.*, 2002). Exposés à de froides températures, les animaux perdent de la chaleur par évapotranspiration, par conduction lorsqu'ils sont en contact avec une surface froide, par convection lorsqu'ils sont exposés au vent et par radiation, surtout la nuit (figure I.4). Pour minimiser les pertes et maximiser les gains de chaleur, les animaux peuvent ajuster leur comportement afin de moduler les échanges de chaleur avec l'environnement. Par exemple, ils peuvent sélectionner des micro-habitats à l'abri du vent pour réduire les pertes de chaleur par convection (Marsh & Dawson, 1989; Scholander *et al.*, 1950b; Wolf *et al.*, 1996) ou ils peuvent s'exposer au soleil pour augmenter les apports de chaleur par radiation (Doucette & Geiser, 2008; Geiser & Drury, 2003).

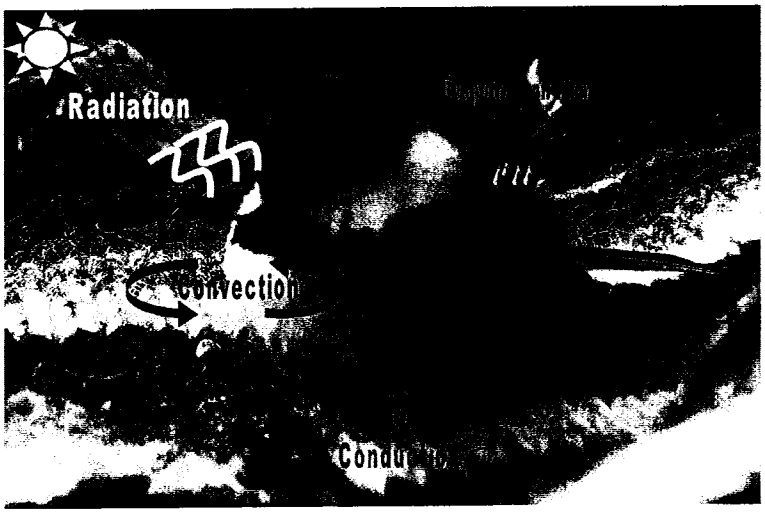

Figure I.4 Echanges de chaleur entre un animal et son environnement. Les transferts de chaleur se font par conduction, convection, évapotranspiration et radiation. © Magali Petit.

I.4.3 Les réponses physiologiques aux froides températures

Les animaux peuvent aussi limiter les pertes de chaleur par des ajustements physiologiques. Par exemple, en augmentant leur isolation thermique (*e.g.* par piloérection) (Marsh & Dawson, 1989; Scholander *et al.*, 1950b; Swanson, 1991a) et en augmentant la vasoconstriction des extrémités (Marsh & Dawson, 1989). De plus, toute activité produisant de la chaleur, la chaleur générée lors de la locomotion ou de la digestion participe aussi au maintien de la température corporelle chez les organismes endothermes, c'est de la thermorégulation par substitution (Cooper & Sonsthagen, 2007).

Lorsque les ajustements comportementaux et la thermorégulation par substitution ne suffisent plus à compenser les pertes de chaleur, les endothermes utilisent principalement la thermogénèse par frissonnement pour produire activement de la chaleur et maintenir leur température corporelle. La thermogénèse par frissonnement représente la première source de chaleur endogène chez tous les vertébrés endothermes (Hohtola, 2002). En réponse au froid, le système nerveux somatique déclenche des contractions asynchrones et soutenues des muscles squelettiques antagonistes. Puisque les contractions sont aléatoires, le frissonnement permet aux animaux de produire de la chaleur, sans toutefois générer de mouvement (Hohtola, 2004).

Un animal exposé à des températures de plus en plus froides augmente ainsi sa production de chaleur jusqu'à atteindre sa capacité thermogénique maximale (Msum, pour « summit metabolic rate », figure I.3). Ensuite, si la température ambiante continue de diminuer, la thermogénèse ne suffit plus à compenser les pertes de chaleur et l'individu entre en hypothermie, ce qui se traduit par une diminution du métabolisme. De ce fait, le Msum correspond au métabolisme maximal d'un individu exposé au froid (Dawson & Smith, 1986; Marsh & Dawson, 1989). Le Msum est mesuré en conditions expérimentales et est généralement atteint à des températures plus froides que celle rencontrées en nature. Cependant, parce que le Msum est corrélé à la tolérance des individus au froid (Swanson, 1990a; Swanson, 2001; Swanson & Liknes, 2006), c'est un indice de la capacité thermogénique couramment utilisé dans les études sur la thermorégulation (McKechnie & Swanson, 2010; Swanson, 2010; Swanson *et al.*, 2012).

I.5 L'acclimatation hivernale chez les endothermes de petite taille: connaissances actuelles et objectifs de la thèse

L'acclimatation hivernale chez les endothermes de petite taille est généralement caractérisée, relativement à l'été, par un accroissement de la taille des organes (Liu & Li, 2006; Zheng *et al.*, 2008), par une augmentation du métabolisme de base et de la capacité thermogénique (Cooper & Swanson, 1994; Swanson & Vézina, 2015; Zhao *et al.*, 2010) ainsi que par une hausse de l'activité des hormones et des enzymes mises en jeu dans le catabolisme des lipides, des glucides et des protéines (Dawson *et al.*, 1992; Liknes & Swanson, 2011a). Ainsi, pour affronter les températures hivernales, ces animaux ajustent leur physiologie (*e.g.* activité enzymatique) (Liknes & Swanson, 2011a) et leur morphologie (*e.g.* taille des organes) (Liknes & Swanson, 2011b).

Bien que l'acclimatation hivernale soit étudiée depuis les années 50 (Scholander *et al.*, 1950a), notre compréhension de ce phénomène est encore à développer. En effet, nos connaissances actuelles se limitent surtout aux ajustements saisonniers et nous n'avons que peu d'informations sur la capacité des endothermes à ajuster leur physiologie sur une courte période de temps. Pour approfondir nos connaissances sur la capacité des endothermes de petite taille à ajuster leur physiologie et leur morphologie aux variations de conditions

hivernales, nous avons analysé la capacité d'ajustement de la mésange à tête noire (*Poecile atricapillus*). Cette thèse regroupe cinq chapitres mettant en évidence les patrons d'ajustements métaboliques intra-saisonniers (***chapitre 1***), les causes exogènes de ces ajustements métaboliques (***chapitre 2***), les mécanismes endogènes qui permettent aux animaux de réaliser ces ajustements (***chapitre 3 et 4***) ainsi que les conséquences des ajustements phénotypiques hivernaux sur la valeur sélective des individus (***chapitre 5***).

I.5.1 Les patrons d'ajustements métaboliques intra-saisonniers

Les changements climatiques sont caractérisés par une augmentation des événements météorologiques extrêmes (*e.g.* innondation, sécheresse) (Easterling *et al.*, 2000). Ces variations environnementales à court-terme peuvent affecter la démographie, la phénologie (Forcada *et al.*, 2008) et la variabilité génétique des espèces (Canale & Henry, 2010) en perturbant, par exemple, les dates de reproduction (Laaksonen *et al.*, 2006; Van Der Jeugd *et al.*, 2009) ou en favorisant les génotypes associés aux phénotypes les plus flexibles (Canale & Henry, 2010). D'après les modèles climatiques, les événements extrêmes sont prévus pour être plus fréquents, notamment dans les régions à hautes latitudes (IPCC, 2007), surtout durant la saison hivernale (DesJarlais *et al.*, 2010). Or, une augmentation de la stochasticité des conditions hivernales devrait faire de l'hiver, une saison déjà énergétiquement contraignante en région tempérée froide, une étape du cycle annuel encore plus difficile à affronter. Néanmoins, grâce à leur capacité à ajuster rapidement et de façon réversible leur physiologie (*i.e.* flexibilité phénotypique, Piersma & Drent, 2003), les animaux pourraient être en mesure de faire face, jusqu'à une certaine limite, à ces variations météorologiques imprévisibles (Canale & Henry, 2010). Afin de comprendre l'effet des fluctuations hivernales sur la physiologie des individus et la dynamique des populations (Vedder *et al.*, 2013), il apparaît donc primordial d'étudier la capacité des espèces endothermes résidant en régions tempérées froides de s'ajuster aux variations rapides de leur environnement.

Nos connaissances sur l'acclimatation hivernale sont encore très limitées. En effet, si les ajustements au froid sont étudiés depuis des décennies (Scholander et al., 1950c), la plupart des études sur l'acclimatation hivernale n'ont fait qu'analyser les variations inter-saisonnières (entre été et hiver) de paramètres physiologiques tels que le BMR ou le Msum (Cooper, 2002;

Cooper & Swanson, 1994; Liknes & Swanson, 1996; Swanson, 1991a). Ainsi, bien que de récentes études se soient intéressées aux variations entre les quatre saisons (Zheng et al., 2008), les études sur l'acclimatation hivernale n'ont pour l'instant pas la résolution temporelle nécessaire pour mettre en évidence les ajustements potentiellement utilisés par les endothermes pour répondre aux variations rapides de l'environnement hivernal (McKechnie, 2008; McKechnie & Swanson, 2010). A notre connaissance, seules deux études (Broggi *et al.*, 2007; Swanson & Olmstead, 1999) ont analysé les ajustements à court terme de la performance métabolique hivernale en conditions naturelles. Swanson & Olmstead (1999), avec des populations de mésanges à tête noire, de juncos ardoisés (*Junco hyemalis*) et de bruants hudsoniens (*Spizella arborea*), et Broggi *et al.* (2007), avec une population de mésanges charbonnières (*Parus major*), ont observé que des oiseaux de petite taille augmentaient leur performance métabolique durant l'hiver (*i.e.* en quelques semaines) en réponse à la diminution de la température ambiante. Basé sur ces deux études, il semble que la température ambiante ait un effet proximal sur la performance métabolique hivernale. Nous pouvons donc supposer que les endothermes de petite taille ajustent leur métabolisme au cours de l'hiver et atteignent leur métabolisme maximal durant la période la plus froide de l'hiver. Cependant, aucune étude n'a encore mis en évidence le patron d'ajustement du métabolisme hivernal chez les espèces résidant sous les hautes latitudes. De plus, bien que les ajustements métaboliques inter-saisonniers soient interprétés comme des exemples de flexibilité phénotypique (Liknes & Swanson, 2011a; Vézina *et al.*, 2011), nous manquons de données intra-individuelles pour supporter cette assertion.

Avec le ***chapitre 1***, nous avons donc analysé les variations du BMR et du Msum en conditions naturelles chez des mésanges à tête noire capturées plusieurs fois au cours de l'hiver afin 1) de déterminer le patron hivernal du BMR et du Msum au sein de la population et 2) d'établir si ce patron reflète effectivement la flexibilité phénotypique individuelle.

I.5.2 Les causes exogènes des ajustements hivernaux

L'hiver sous les hautes latitudes représente un défi énergétique pour les espèces endothermes qui n'hibernent pas (Chappell, 1980; Cooper, 2000) puisqu'elles doivent faire face à un apport alimentaire réduit (Swanson, 2010) alors que les besoins énergétiques pour la thermorégulation augmentent (Liknes & Swanson, 1996). Les animaux vivant dans ces

régions sont donc soumis à des contraintes environnementales qui les forcent à ajuster leur physiologie pour survivre à la saison hivernale. Ils sont par conséquent de bons modèles pour étudier les ajustements phénotypiques en fonction des variations naturelles de conditions environnementales. La capacité des animaux à ajuster leur phénotype à leur environnement peut être étudiée au niveau individuel et populationnel (Brommer, 2013; Charmantier *et al.*, 2008) par les normes de réaction qui décrivent la flexibilité d'un paramètre le long d'un gradient environnemental (McKechnie, 2008; Nussey *et al.*, 2007; Schlichting & Pigliucci, 1998). Dans le cas où les individus d'une population exprimeraient une flexibilité phénotypique différente et où la flexibilité phénotypique serait soumise à la sélection naturelle (Scheiner & Lyman, 1991), la stochasticité environnementale favoriserait la survie des individus les plus flexibles, conduisant à une microévolution de la population (Nussey *et al.*, 2007). A l'opposé, si les individus exprimaient un phénotype moyen similaire et une flexibilité comparable, toute la population répondrait de façon semblable aux variations de leur environnement (Nussey et al., 2007). Afin de comprendre les effets des variations environnementales sur la dynamique des populations, il est donc important d'étudier la capacité des endothermes à ajuster leur physiologie en fonction du gradient naturel des conditions environnementales. Or, bien que les normes de réaction soient souvent étudiées chez les ectothermes (Angilletta, 2009), peu d'études similaires ont été effectuées chez des endothermes.

Si quelques études reportent des variations phénotypiques pour des périodes de moins de six mois (Bozinovic *et al.*, 2007; Liknes & Swanson, 2011b; Zhao *et al.*, 2010; Zheng *et al.*, 2008), la plupart des études sur l'acclimatation hivernale ne montrent qu'une augmentation du métabolisme entre l'été et l'hiver (Liknes & Swanson, 1996; Zhao *et al.*, 2010). De la même façon, au lieu de s'intéresser à la flexibilité métabolique le long d'un gradient continu de températures, les études expérimentales portant sur les ajustements métaboliques utilisés par les endothermes pour répondre aux variations thermiques n'utilisent généralement que des changements discrets de températures (*i.e.* les individus ne sont soumis qu'à quelques traitements thermiques fixes) (Maggini & Bairlein, 2013; Williams & Tieleman, 2000). Ces approchent qui limitent nos connaissances à des états physiologiques stables, procurent peu d'information sur la dynamique des ajustements physiologiques. De plus, les ajustements métaboliques pouvant être limités par la physiologie ou la morphologie des individus, les normes de réaction du métabolisme hivernal pourraient être non-linéaires (*i.e.* le métabolisme

atteindrait un plateau) (McKechnie, 2008). Or, les quelques études qui ont analysé la relation entre le métabolisme et les variations météorologiques ont testé pour un effet linéaire de la température sur le métabolisme (Jackson et al., 2001; Swanson & Olmstead, 1999 mais voir Broggi *et al.*, 2007). Notre connaissance de la capacité des endothermes non migrateurs à ajuster leur métabolisme aux variations de température reste donc limitée. Enfin, bien que la température ambiante affecte indubitablement le métabolisme des endothermes de petite taille (McKechnie & Swanson, 2010; Swanson & Olmstead, 1999), les mécanismes de transfert de chaleur impliquent aussi d'autres facteurs environnementaux tels que la radiation solaire, l'humidité ou la vitesse de vent (Bakken *et al.*, 1991; Hayes & Gessaman, 1980; Robinson *et al.*, 1976; Walsberg & Wolf, 1995). Du fait que la majorité des études sur l'acclimatation hivernale ne considèrent que la température comme cause exogène de l'augmentation du métabolisme hivernal, notre compréhension des effets des variations à court terme des conditions météorologiques hivernales sur la physiologie des endothermes de petite taille est encore partielle.

Avec le ***chapitre 2***, nous avons donc analysé les normes de réaction du BMR et du Msum en fonction des variations naturelles des conditions météorologiques subies par une population de mésanges à tête noire afin 1) de déterminer l'influence des différents paramètres météorologiques sur la performance métabolique et 2) de mettre en évidence le patron des normes de réaction du BMR et du Msum.

I.5.3 Les mécanismes endogènes des ajustements hivernaux

Le BMR est interprété comme étant la dépense énergétique nécessaire au maintien de l'activité minimale des organes et des muscles chez un individu au repos (Chappell *et al.*, 1999) tandis que le Msum est interprété comme la dépense énergétique maximale d'un individu lors de la thermogénèse par frissonnement (Swanson *et al.*, 2013). Considérant que les variations du métabolisme reflètent un changement de la taille et/ou de l'activité des organes et des muscles (Burness *et al.*, 1998; Chappell *et al.*, 1999; Daan *et al.*, 1990; Piersma & Van Gils, 2010; Weber & Piersma, 1996), l'augmentation du BMR entre l'été et l'hiver est généralement perçue comme résultant d'une modification de la composition corporelle (Swanson, 2010; Vézina *et al.*, 2006). Par exemple, Williams et Tieleman (2000) ont observé que des sirlis du desert (*Alaemon*

alaudipes) en captivité augmentaient aussi bien leur BMR que la masse de leurs estomac, intestin, foie et reins lorsqu'ils étaient acclimatés au froid. De leurs côtés, Zheng *et al* (2008) ont observé que les moineaux friquets (*Passer montanus*) capturés en hiver avaient un BMR et une masse d'intestin, de foie et de cœur plus élevée que leurs congénères capturés en été. Ces données suggèrent donc une contribution prédominante des organes digestifs et du cœur à l'augmentation du BMR hivernal (Swanson, 2010). De la même façon, des études en milieu naturel (Cooper, 2002; O'Connor, 1995; Swanson *et al.*, 2013) et en captivité (Vézina *et al.*, 2011; Vézina *et al.*, 2007) ont observé une augmentation parallèle de la capacité thermogénique et de la taille des muscles pectoraux. Ainsi, l'augmentation du Msum entre l'été et l'hiver est généralement perçue comme étant liée à une augmentation de la masse musculaire, notamment des muscles pectoraux (Cooper, 2002; Saarela & Hohtola, 2003; Swanson *et al.*, 2013; Vézina *et al.*, 2007), ce qui est d'ailleurs supporté par une récente étude de Swanson *et al.* (2013) sur les chardonnerets jaunes (*Spinus trisis*). Cependant, les évidences actuelles d'un lien entre le métabolisme et la masse ou l'activité des tissus ne sont basées que sur l'utilisation d'analyses univariées, lorsqu'elles ne reposent pas tout simplement sur l'observation d'une augmentation concomitante du métabolisme et de la masse de quelques organes. Ainsi, à notre connaissance, aucune étude n'a encore pu mettre en évidence la contribution relative des différents organes à la variation du métabolisme dans un contexte d'acclimatation hivernale.

Puisque les études sur les ajustements inter-saisonniers évoquent un lien entre l'augmentation du métabolisme et un accroissement des organes et des muscles entre l'été et l'hiver, les ajustements de la performance métabolique mis en place par les endothermes de petite taille au cours de l'hiver pourraient aussi être liés à des modifications à court terme de la masse des organes et des muscles. Or, si certaines études ont mis en évidence une modification de la composition corporelle entre les quatre saisons (Liknes & Swanson, 2011b; Liu & Li, 2006; Zheng *et al.*, 2008), nous ne connaissons pas la capacité des endothermes à ajuster leur composition corporelle sur une courte période au cours de l'hiver.

La capacité maximale de transport de l'oxygène, mesurée *via* l'hématocrite (*i.e.* la proportion d'hématies dans le sang), contribue à supporter l'activité métabolique totale (Burness *et al.*, 1998; Calbet *et al.*, 2006; Carpenter, 1975; Hammond *et al.*, 2000), dont la thermogénèse (Carey & Morton, 1976; Swanson, 1990b). Par exemple, Swanson (1990a, 1990b) a observé à la fois

une augmentation de l'hématocrite et du Msum chez les juncos ardoisés capturés en hiver, relativement à ceux capturés en été. L'augmentation hivernale de l'hématocrite est donc interprétée comme une réponse aux besoins élevés en oxygène liés à l'augmentation de la thermogénèse par frissonnement en hiver (Carey & Morton, 1976; Swanson, 1990b). Cependant, tout comme le lien entre le Msum et la taille des muscles pectoraux n'est pour l'instant que corrélatif et reste à être démontré expérimentalement, le lien fonctionnel entre le Msum et l'hématocrite doit lui aussi à être mis en évidence.

Dans un premier temps, avec le ***chapitre 3***, nous avons réduit la surface de portance des ailes et de la queue (Ardia & Clotfelter, 2007; Harding *et al.*, 2009b; Sanz *et al.*, 2000) de mésanges à tête noire capturées en hiver afin de les forcer à augmenter la taille de leurs muscles pectoraux (Lind & Jakobsson, 2001). La capacité thermogénique étant censée être liée à la taille des muscles pectoraux (Swanson *et al.*, 2013; Vézina *et al.*, 2007) et au transport de l'oxygène (Carey & Morton, 1976; Swanson, 1990b), cette étude nous a permis de tester expérimentalement la relation entre la taille des muscles pectoraux, la capacité thermogénique et l'approvisionnement en oxygène des oiseaux de petite taille hivernant en régions tempérées froides.

Ensuite, avec le ***chapitre 4***, nous avons analysé les ajustements de la masse des organes et des muscles ainsi que ceux du BMR et du Msum chez des mésanges à tête noire capturées en milieu naturel. Notre but était 1) de mettre en évidence les patrons d'ajustements intra-hivernaux de la composition corporelle et de la performance métabolique et 2) de déterminer la contribution de chaque tissu à la variation du métabolisme au sein de la saison hivernale.

I.5.4 Les conséquences des ajustements hivernaux sur la valeur sélective

Pour affronter les conditions hivernales en régions tempérées froides, les endothermes de petite taille augmentent leur masse corporelle (Vézina *et al.*, 2006; Williams & Tieleman, 2000), leur réserve lipidique (Cooper, 2002; Swanson, 1991a), leur performance métabolique (Cooper & Swanson, 1994) ainsi que leur capacité de transport de l'oxygène (Swanson, 1990b). Ces études suggèrent donc que les oiseaux ayant les plus gros muscles, les réserves lipidiques les plus importantes et la performance métabolique la plus élevée seraient les mieux équipés pour affronter les conditions hivernales et, conséquemment, auraient une

meilleure survie hivernale et à long terme que leurs congénères exprimant une moins bonne condition physiologique (Brodin, 2007; Pravosudov & Lucas, 2001; Verhulst *et al.*, 2004). Cependant, selon le principe de l'allocation des ressources (Weiner, 1992), les individus faisant face à des conditions énergétiquement difficiles, tel que l'hiver, doivent utiliser des compromis physiologiques. Cela permet de maintenir les activités principales (*e.g.* thermogénèse) aux dépens des autres fonctions physiologiques (*e.g.* système immunitaire) (Ardia & Clotfelter, 2007; Buehler *et al.*, 2009; Wiersma *et al.*, 2004). Dans ce contexte, l'énergie dépensée par les animaux durant l'hiver pour maintenir leur température corporelle et leur prise alimentaire pourrait, si elle s'avérait trop élevée, compromettre leur survie hivernale *via,* par exemple, une détérioration de leurs défenses immunitaires, et conduire à des effets sur le long terme néfastes pour leur valeur sélective (Fretwell, 1972; Saino *et al.*, 2004). Par conséquent, pour déterminer l'effet des conditions hivernales sur la dynamique des populations, il nous faut comprendre la relation entre le phénotype hivernal et la survie des individus. Or, bien qu'une relation positive entre les paramètres physiologiques et la survie hivernale soit souvent supposée (Nzama *et al.*, 2010; Sears *et al.*, 2006; Swanson & Olmstead, 1999) et ait été observée chez quelques mammifères de petite taille (Boratyński *et al.*, 2010; Jackson *et al.*, 2001), elle reste à démontrer chez les oiseaux en conditions naturelles (Swanson & Vézina, 2015).

Avec le ***chapitre 5***, nous avons utilisé un protocole de capture-marquage-recapture (CMR) sur une population de mésanges à tête noire durant trois hivers afin d'analyser l'effet de la condition physiologique hivernale, mesurée *via* la masse corporelle, l'hématocrite et la performance métabolique, à la fois sur leur survie hivernale et la survie à long terme.

I.6 Méthodologie générale

La méthodologie spécifique à chaque étude est détaillée dans le chapitre approprié. D'une façon générale, nous avons appliqué trois protocoles principaux: 1) un protocole de capture-marquage-recapture (CMR) réparti de septembre 2009 à décembre 2011, 2) un protocole expérimental utilisé durant le deuxième hiver et 3) un protocole de capture-sacrifice-dissection réparti sur la deuxième année.

I.6.1 Modèle d'étude : la mésange à tête noire (*Poecile atricapillus*)

Les oiseaux ont un métabolisme plus élevé que les mammifères de même taille (Hulbert *et al.*, 2007) et, du fait de leur rapport surface/volume élevé, les espèces de petite taille sont plus sensibles aux pertes de chaleur que les espèces de grande taille. Ainsi, les oiseaux de petite taille sont de parfaits modèles pour étudier les ajustements métaboliques utilisés par les espèces résidant en régions tempérées froides pour affronter les conditions hivernales.

Pour ces études nous avons choisi de travailler avec la mésange à tête noire (*Poecile atricapillus*) (figure I.5), un petit (9-14 g) passereau non migrateur, territorial et largement distribué en Amérique du Nord (figure I.6) (Sharbaugh, 2001; Smith, 1991). Le cycle annuel de la mésange à tête noire comprend trois périodes : 1) la saison de reproduction de fin avril à mi-juillet (Odum, 1941a, 1941b), 2) la mue de juillet à août (Desrochers *et al.*, 1988; Meigs *et al.*, 1983) et 3) la saison hivernale de septembre à avril (Odum, 1942).

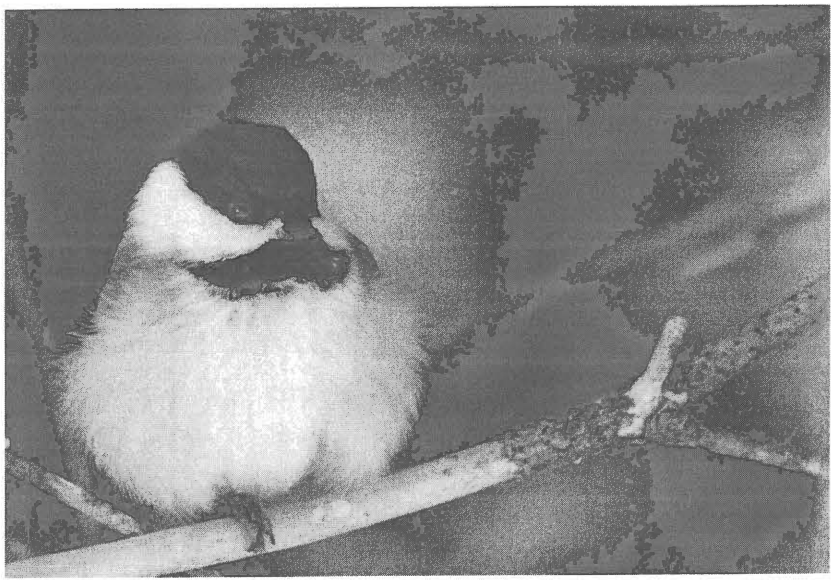

Figure I.5 Mésange à tête noire en hiver, forêt Macpès. © François Vézina.

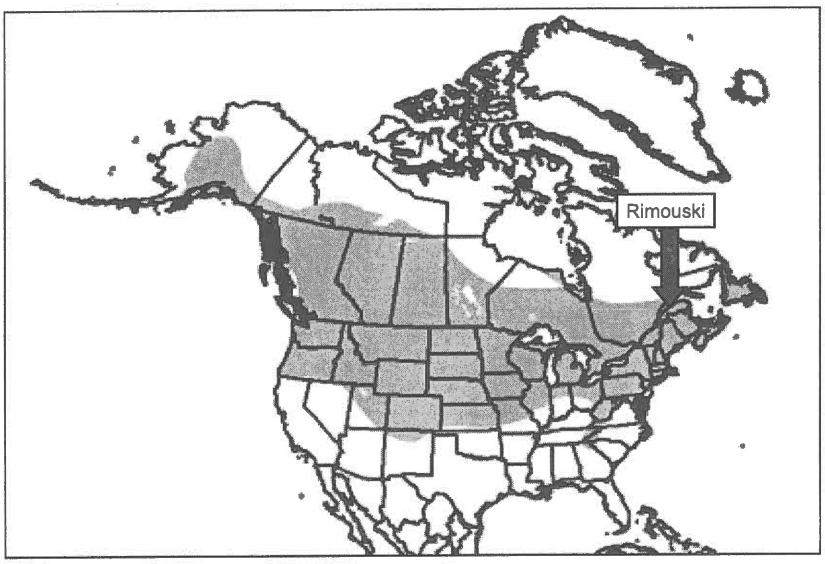

Figure I.6 Distribution de la mésange à tête noire. La répartition de la mésange à tête noire s'étend entre 37°N (Colorado) et 67°N (Alaska) (gris). Au site d'étude (flèche rouge), à Rimouski (Québec, Canada), les mésanges sont près de la limite nord-est de leur aire de distribution. (Source Environnement Canada : http://www.ec.cg.ca).

En hiver, les mésanges vivent en groupes de 3 à 15 individus (Desrochers *et al.*, 1988; Odum, 1942) organisés selon une hiérarchie sociale linéaire, sur un territoire de 10 à 20 ha (Desrochers *et al.*, 1988; Smith, 1991; Turcotte & Desrochers, 2008). Durant cette période, le régime alimentaire des mésanges est constitué jusqu'à 50% de matière végétale (Smith, 1993). Au printemps, les groupes sociaux éclatent, les couples reproducteurs se forment et occupent des territoires de 1 à 5 ha (Smith, 1991). Durant la saison de reproduction, les mésanges produisent 6 à 8 œufs avec un succès d'envol de 4,5 juvéniles par couvée en moyenne (Kluyver, 1961; Smith, 1967). En été, leur alimentation est principalement constituée d'insectes. L'espérance de vie moyenne des mésanges à tête noire en conditions naturelles est de 2,5 ans (Smith, 1991).

La mésange à tête noire est une espèce de petite taille, son espérance de vie est courte et notre site d'étude est proche de la limite nord-est de l'aire de répartition de l'espèce. Ainsi, la population de mésange à tête noire sélectionnée pour ce projet est idéale pour analyser les ajustements physiologiques utilisés par les endothermes de petite taille confrontés aux variations de leur environnement hivernal ainsi que pour étudier les conséquences de ces ajustements sur la valeur sélective des individus.

I.6.2 Site d'étude

Le site d'étude choisi est à environ 20 km au sud de Rimouski (Québec), dans la forêt d'enseignement et de recherche (FER) Macpès (figure I.7), gérée par le Cégep de Rimouski. Cette forêt, majoritairement mixte, s'étend sur environ 2300 ha. Plusieurs sites de nourrissage ont été installés au sein de la FER afin d'attirer les mésanges à des points précis pour faciliter leur capture et leur suivi. Chaque site, disposé à une distance de 20 m à 100 m des chemins forestiers, était accessible à l'année. Un pavillon de recherche, situé au cœur de la FER, nous a permis d'effectuer toutes les mesures physiologiques dans les 24 heures suivant la capture des individus. La FER Macpès est équipée de trois tours météorologiques enregistrant en continu les données de rayonnement solaire, de vitesse et direction du vent, de pluviométrie, de température de l'air, d'humidité relative, de pression barométrique et d'hauteur de neige (figure I.8).

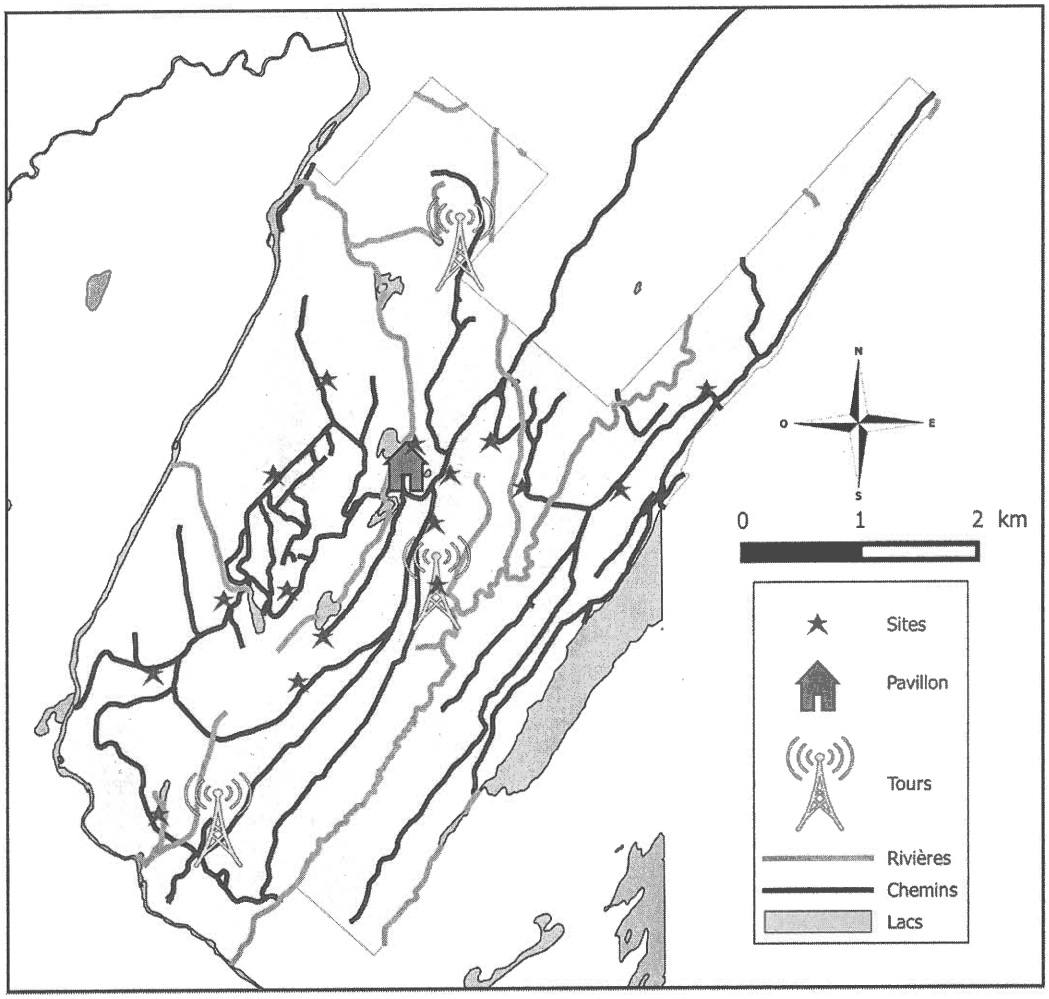

Figure I.7 Forêt d'enseignement et de recherche (FER) Macpès. Sont représentées les trois tours météorologiques (jaune), le pavillon de recherche et les 16 sites de capture (étoiles rouges) utilisés pour cette étude.

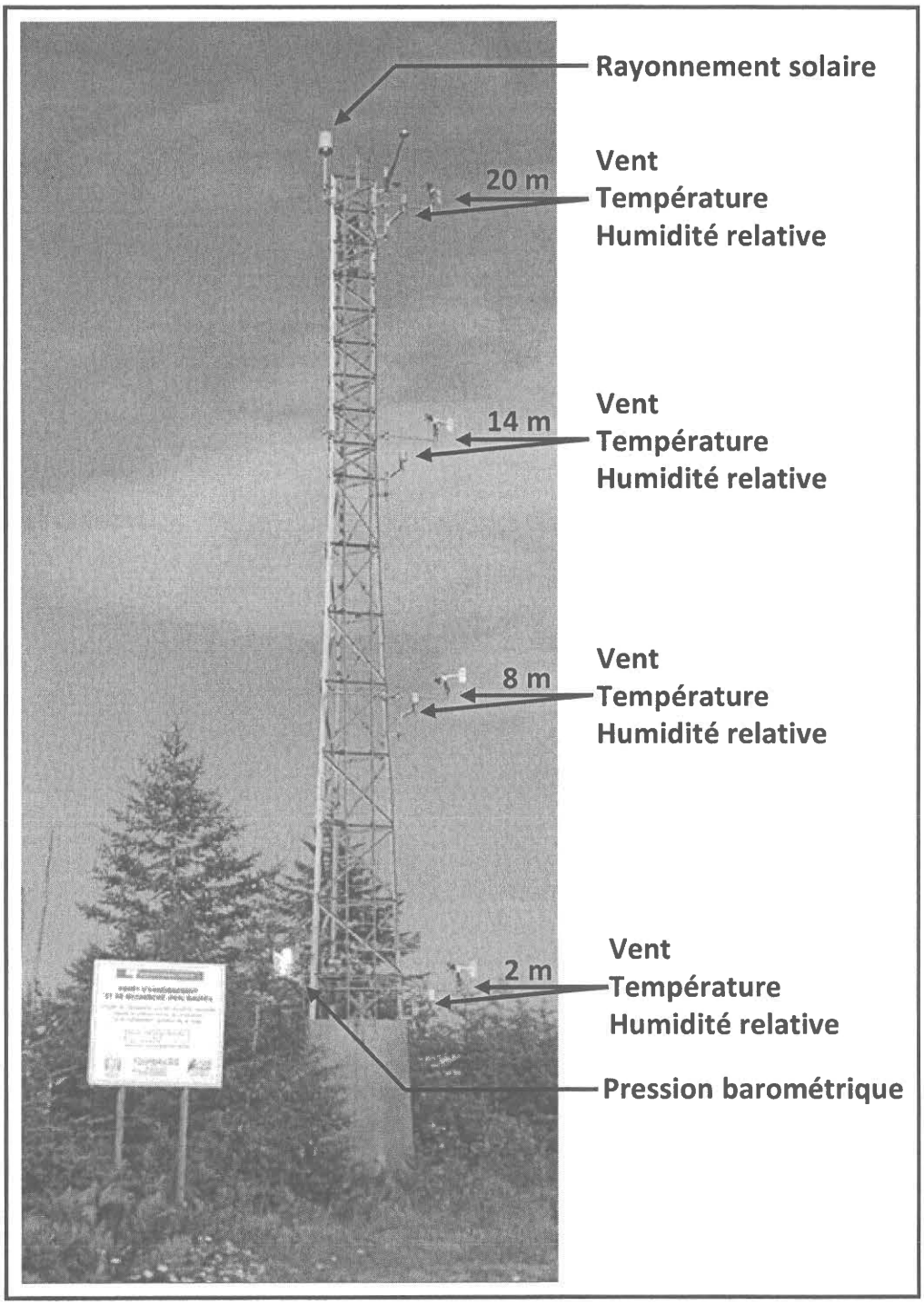

Figure I.8 Tour météorologique de la FER Macpès. © Magali Petit.

I.6.3 Protocole de capture-marquage-recapture (CMR)

I.6.3.1 Méthodes de captures

Les captures de mésanges à tête noire ont été effectuées de septembre 2009 à mars 2010, d'août 2010 à mars 2011 ainsi qu'en août 2011 (***chapitres 1 à 5***). Afin de faciliter les captures, 16 sites de nourrissage ont été installés au sein de la FER (figure I.7). Chaque site incluait trois mangeoires remplies de graines de tournesol et deux poteaux en bois. Nous utilisions des trappes (15 cm x 15 cm x 15 cm) à porte coulissante posées sur les poteaux (figure I.9) afin de capturer les mésanges et de pouvoir les manipuler aussitôt. En novembre et décembre 2011, nous avons effectué des séances d'observation standardisées afin de relocaliser les individus et d'estimer le taux de survie de la population (***chapitre 5***).

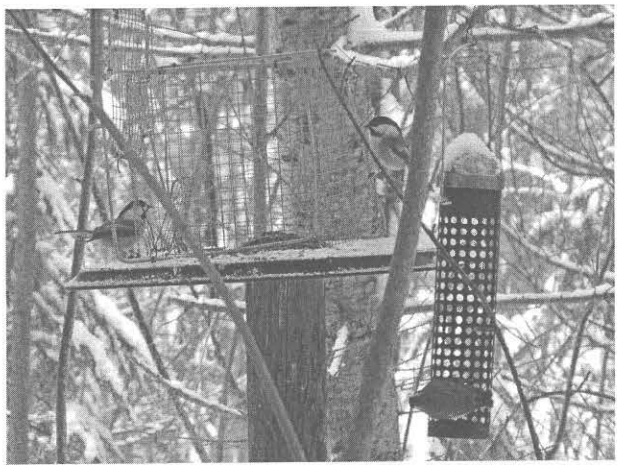

Figure I.9 Sites de capture. Les mésanges, attirées par les graines de tournesol, entrent dans la trappe et y sont piégées par la porte coulissante. © Magali Petit.

I.6.3.2 Mesures morphologiques et prélèvements sanguins

Tout nouvel oiseau capturé était bagué d'une bague de métal du service canadien de la faune ainsi que d'une combinaison de trois bagues de couleur permettant l'identification à distance (***chapitres 1 à 5***). A chaque capture, les mésanges étaient pesées, mesurées et le volume de gras furculaire (figure I.10a) ainsi que la taille des muscles pectoraux (figure I.10b) étaient estimés. Un prélèvement sanguin était aussi effectué au niveau de la

veine alaire. Ensuite, les individus étaient amenés au pavillon de recherche afin de procéder aux mesures de métabolisme par respirométrie (figure I.11).

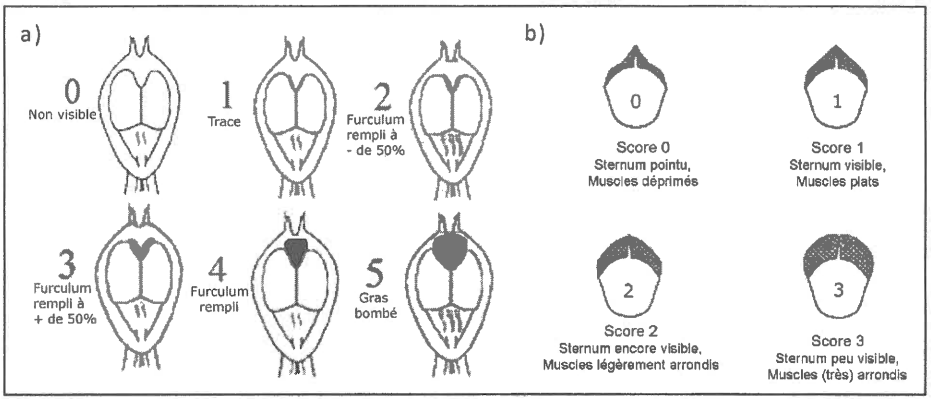

Figure I.10 Mesures morphométriques. a) Indice de gras furculaire sur une échelle de 0 à 5 (d'après Gosler, 1996). b) Taille des muscles pectoraux sur une échelle de 0 à 3 (d'après Busse, 2000).

I.6.3.3 Respirométrie

Toutes les études réalisées pour cette thèse (***chapitres 1 à 5***) comprennent des mesures de respirométrie. Dans tous les cas, quatre oiseaux capturés le matin (entre 08:00 et 12:00) étaient ramenés au pavillon afin de mesurer leur métabolisme par respirométrie. L'après-midi, les mesures de Msum étaient effectuées sur des oiseaux exposés au froid. Durant la nuit, les mesures de BMR étaient effectuées sur des oiseaux maintenus à thermoneutralité. Le lendemain matin, les oiseaux étaient relâchés sur leur territoire.

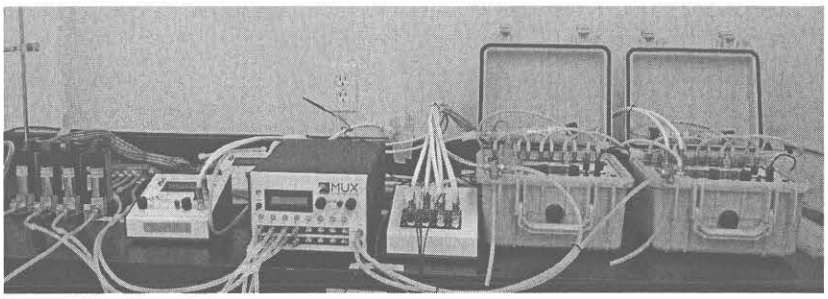

Figure I.11 Système de respirométrie utilisé pour mesurer le métabolisme des mésanges à la FER Macpès. © Magali Petit.

1.6.4 Protocole expérimental : le « feather clipping »

Afin de tester la relation entre la masse musculaire et la capacité thermogénique des oiseaux (***chapitre 3***), nous avons manipulé la surface de portance des ailes et de la queue pour affecter la taille des muscles pectoraux des mésanges durant le deuxième hiver du projet (octobre 2010 à mars 2011). Cette méthode consiste à couper certaines plumes des ailes et de la queue afin de forcer les oiseaux à compenser la perte de surface de portance par une augmentation de la taille des muscles pectoraux. Pour notre étude, nous avons choisi de couper les plumes le long du rachis afin d'éviter que les oiseaux ne régénèrent leur plumes durant l'expérience et que l'on perde l'effet de la manipulation (figure I.12).

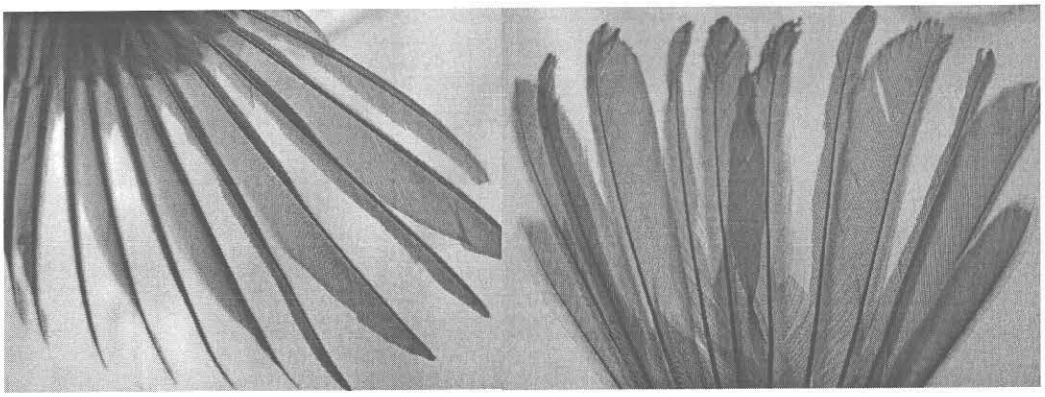

Figure I.12 Aile et queue dont les primaires (P1, P3, P5, P7 et P9) et les rectrices (R2 et R4) furent coupées le long du rachis. © Magali Petit.

1.6.5 Protocole de capture-sacrifice-dissection

Pour tester la relation entre la composition corporelle et la performance métabolique, nous avons utilisé un protocole de capture-sacrifice-dissection au cours de la deuxième année du projet (***chapitre 4***). Pour ce protocole, au lieu d'être relâchés après la mesure du BMR, les oiseaux étaient sacrifiés et disséqués afin de mesurer la masse de leurs organes. Ensuite, les organes étaient séchés par lyophilisation et les lipides en étaient extraits par dissolution dans de l'éther de pétrole en utilisant un appareil de soxhlet afin d'analyser l'influence de la masse maigre des organes sur le BMR et le Msum.

CHAPITRE I

La flexibilité intra-saisonnière de la performance métabolique révèle un découplage entre le métabolisme basal et la capacité thermogénique chez les oiseaux

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Intra-seasonal flexibility in avian metabolic performance highlights the uncoupling of basal metabolic rate and thermogenic capacity

Magali Petit[1, 2*]; Agnès Lewden[1], François Vézina[1, 2]

[1]Département de biologie, chimie et géographie, Université du Québec à Rimouski, Rimouski (QC), Canada. Groupe de recherche sur les environnements nordiques BORÉAS, Rimouski, (QC), Canada. [2]Centre d'Études Nordiques, Québec (QC), Canada. Centre de la Science de la Biodiversité du Québec, Montréal (QC), Canada.

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Contributions - Conception de l'expérience: MP, AL, FV ; Réalisation de l'expérience: MP, AL. ; Analyse des données: MP ; Financement: FV ; Rédaction : MP, FV.

1.1 Résumé

Sous les latitudes nordiques, les scenarios climatiques prévoient une augmentation de la fréquence et de l'amplitude des événements météorologiques extrêmes pendant l'hiver, une stochasticité hivernale que les organismes sont censés supporter grâce à leur flexibilité phénotypique. Les petites espèces aviaires résidant dans ces environnements montrent une acclimatation saisonnière caractérisée par une augmentation de leur métabolisme de base (BMR) et de leur capacité thermogénique maximale (Msum). Cependant, nous ne connaissons pas les patrons intra-saisonniers de la performance métabolique ni si les variations à l'échelle populationnelle reflètent la flexibilité individuelle. Nous avons donc réalisé une étude sur la variation intra-saisonnière de la performance métabolique mesurée chez la mésange à tête noire (*Poecile atricapillus*) aux niveaux populationnel et individuel. Les résultats confirment que les patrons pour la population sont représentatifs de la flexibilité individuelle. Tel que prévu, les données montrent une augmentation du BMR (6%) et du Msum (34%) entre l'été et l'hiver mais aussi, et surtout, que ces deux paramètres suivent des patrons d'ajustement différents. Alors que l'augmentation du BMR ne commence qu'en novembre pour revenir à un niveau estival en mars, le Msum atteint 20% de son augmentation inter-saisonnière dès octobre et reste élevé jusqu'à mars. Bien que ces deux paramètres covarient au cours de l'année, le découplage temporel observé entre les ajustements du BMR et du Msum au cours de l'hiver suggère qu'ils sont influencés par des contraintes différentes et que le BMR et le Msum n'ont pas de lien fonctionnel.

Mots-clefs: flexibilité phénotypique ; BMR ; Msum ; acclimatation hivernale ; variation individuelle ; *Poecile atricapillus*.

1.2 Abstract

Stochastic winter weather events are predicted to increase in occurrence and amplitude at northern latitudes and organisms are expected to cope through phenotypic flexibility. Small avian species wintering in these environments show acclimatization where basal metabolic rate (BMR) and maximal thermogenic capacity (Msum) are typically elevated. However, little is known on intra-seasonal variation in metabolic performance and on how population trends truly reflect individual flexibility. Here, we report intra-seasonal variation in metabolic parameters measured at the population and individual levels in Black-capped chickadees (*Poecile atricapillus*). Results confirmed that population patterns indeed reflect flexibility at the individual level. They showed the expected increase in BMR (6%) and Msum (34%) in winter relative to summer but also, and most importantly, that these parameters changed differently through time. BMR began its seasonal increase in November while Msum had already achieved more than 20% of its inter-seasonal increase by October, and declined to its starting level by March while Msum remained high. Although both parameters co-vary on a yearly scale, this mismatch in the timing of variation in winter BMR and Msum likely reflects different constraints acting on different physiological components and therefore suggests a lack of functional link between these parameters.

Keywords: Phenotypic flexibility; BMR; Msum; winter acclimatization; individual variation; *Poecile atricapillus*.

1.3 Introduction

With global changes comes a higher frequency of unpredictable weather events (Easterling *et al.*, 2000). These short-term environmental fluctuations can affect animal species through changes in demography, phenology (Forcada *et al.*, 2008) and genetic variability (Canale & Henry, 2010), for example by disturbing the timing of reproduction (Laaksonen *et al.*, 2006; Van Der Jeugd *et al.*, 2009) or by favouring genotypes that produce flexible rather than stable phenotypes (Canale & Henry, 2010). This may be particularly important for northern latitude species where warming is accelerated relative to lower latitudes (IPCC, 2007), especially in winter (DesJarlais *et al.*, 2010), and where the occurrence and amplitude of short term stochastic events are predicted to increase (IPCC, 2007). Phenotypic flexibility, the rapid and reversible transformations of phenotypic traits that allow adult individuals to adjust their behaviour and physiology to predictable or stochastic changes in the environment (Piersma & Drent, 2003), should provide a certain capacity to buffer these variations (Canale & Henry, 2010). However, in natural settings, little is known on how animals adjust their phenotype to intra-seasonal changes in ecological conditions (McKechnie, 2008; Swanson & Olmstead, 1999).

Winter at northern latitudes is typically considered a challenging season for resident bird species (Cooper, 2000). Since they remain active throughout the cold season, low ambient temperatures force these animals to increase energy expenditure for thermoregulation (Cooper & Swanson, 1994; Liknes & Swanson, 1996) while short days, snow and ice cover may reduce foraging time and food availability (McNamara *et al.*, 1990; Swanson, 2010). In small bodied species, thermoregulatory constraints are exacerbated because of their large surface area relative to volume, which increases heat loss (McNab, 1971), in addition to their limited ability to carry thick insulative plumage. Small birds therefore use physiological adjustments to improve cold tolerance (Cooper & Swanson, 1994; Swanson, 1991a) and their chances of survival. Seasonal acclimatization is typically associated with a winter increase in metabolism visible in parameters such as basal metabolic rate (BMR; physiological maintenance cost) and summit metabolic rate (Msum; maximal thermogenic capacity) (Cooper & Swanson, 1994; Swanson, 1991a; Zheng *et al.*, 2008). However, although cold acclimatization has been investigated for decades (Scholander *et al.*, 1950c), most field studies lack the required temporal resolution to address questions regarding individual physiological adjustments in response to intra-seasonal variations

in ambient conditions (McKechnie, 2008; McKechnie & Swanson, 2010). Studies typically compare phenotypic traits such as BMR and Msum on a seasonal basis, comparing values from individuals captured in winter with data collected on different individuals captured in summer (Cooper, 2002; Cooper & Swanson, 1994; Liknes & Swanson, 1996; Swanson, 1991a; Zheng *et al.*, 2008).

To face stochastic winter conditions, small birds could use two mutually non-exclusive strategies. First, they could maintain a constant and elevated winter metabolism. This strategy would increase survival during extreme thermal events but it would also presumably be associated with high and often unnecessary maintenance costs. Second, birds could use phenotypic flexibility to rapidly adjust their physiology to prevailing conditions. In this situation, however, individuals may have to invest considerable resources in physiological readjustments (*e.g.* adjustment in organ size, Dekinga *et al.*, 2001). As far as we know, only two studies have provided data on intra-seasonal metabolic changes in response to winter climate variations in small free-living birds (Broggi *et al.*, 2007; Swanson & Olmstead, 1999), both of these reflected a phenotypic flexibility strategy. Swanson and Olmstead (1999) observed that cold ambient temperature was associated with elevated metabolic performance in dark-eyed juncos (*Junco hyemalis*), Black-capped chickadees (*Poecile atricapillus*) and American tree sparrows (*Spizella arborea*). This was visible at the population level on a between-year timescale for BMR and Msum and on a between-month (*i.e.* intra-seasonal) timescale for Msum (there were not enough data for testing the effect on BMR). Furthermore, ambient temperature preceding measurements appeared to affect metabolic performance within relatively short periods, between one and 30 days, depending on the species. Similar findings were reported by Broggi *et al.* (2007) in great tits (*Parus major*) where BMR changed throughout winter and responded to ambient temperature averaged over the preceding week.

If ambient temperature exerts such a proximal effect on winter metabolic performance, one would therefore expect adjustments in metabolic parameters over the course of the season, with peak capacity observable at the coldest time of winter. However, data on the shape of metabolic transformations within seasons are lacking, albeit being called for (McKechnie, 2008; McKechnie & Swanson, 2010). Furthermore, although seasonal changes in metabolic performance are interpreted as a clear and evident example of phenotypic flexibility in response

to winter constraints (Vézina *et al.*, 2011), within individual data to support this statement are still lacking for free-living wintering birds. Our understanding of intra-seasonal and intra-individual variation in metabolic performance of birds wintering at northern latitudes therefore remains poor.

Here, we report results of a study where we followed intra-seasonal changes in metabolic performance over two consecutive winters in a population of Black-capped chickadees from eastern Canada. We measured changes in BMR to assess variations in physiological maintenance costs and we measured changes in Msum to follow adjustments in winter thermogenic capacity. These measurements were also performed in August of each year to obtain a summer reference point for comparison. Our first objective for this part of the study was to determine patterns of variation in BMR and Msum within winter. We expected a gradual increase in metabolic performance beginning in autumn to reach a peak at the coldest of winter (*i.e.* January-February), followed by a gradual decline to reach summer values (Broggi *et al.*, 2007). Our second objective was to confirm that these patterns were also visible within individuals and therefore confirm that observations at the population level reflect individual phenotypic flexibility.

Previous studies suggested a functional link between BMR and Msum (Dutenhoffer & Swanson, 1996; Hinds *et al.*, 1993; Swanson & Olmstead, 1999) but some evidence rather suggests that these variables reflect physiological systems acting independently (McKechnie & Swanson, 2010; Swanson *et al.*, 2012; Vézina *et al.*, 2006). BMR would mainly reflect energy requirements of internal organs (Liknes & Swanson, 2011b; Piersma, 2002; Zheng *et al.*, 2008) while Msum would reflect the size of muscles involved in active shivering (Cooper, 2002; Saarela & Hohtola, 2003; Vézina *et al.*, 2007). Given recent contrasting findings, including in our own model species (Lewden *et al.*, 2012; Swanson *et al.*, 2012), we also had an interest in testing the relationship between BMR and Msum with an extensive dataset.

Metabolic expansibility (ME), the ratio of maximal over minimal metabolic rates (Msum / BMR) (Hinds *et al.*, 1993; Swanson, 2010; Swanson *et al.*, 2012), is interpreted as the capacity of an organism to increase its level of heat production for a given size of metabolic machinery (Arens & Cooper, 2005; Cooper & Swanson, 1994). Therefore, variations in ME should also be

a useful variable to evaluate co-adjustment of physiological maintenance costs and thermogenic capacity. We thus report inter- and intra-individual variation in ME throughout winter.

To meet our objectives, 228 individuals were captured during winters 2010 and 2010-11 and had their metabolic performance measured within the following 24 h. Of this number, 56 individuals were recaptured and remeasured between one and five times within a same winter.

1.4 Material and methods

1.4.1 Capture and handling

This study was carried out within the Forêt d'Enseignement et de Recherche Macpès, Québec, Canada (48°18' N, 68°31' W) between January and March 2010 (n = 56) and from October 2010 to March 2011 (n = 149). Data from summer individuals were collected in August 2010 (n = 12) and 2011 (n = 11). To attract chickadees and facilitate capture, 16 feeding stations were set up within the forest with an average distance between stations of 1.9 km (see Lewden *et al,* 2012, Mandin & Vézina, 2012 for a description of the stations). Feeders were regularly filled with black sunflower seeds. On capture days, feeders were removed and homemade potter traps (15 cm x 15 cm x 15 cm) baited with seeds were placed on a tray installed on a wooden fence pole. All birds were caught during morning (between 08:00 and 13:00) and removed from traps within one minute of capture. Weather stations in the forest (Mandin & Vézina, 2012) recorded temperature data allowing us to track ambient temperatures over the two years of the study.

Birds caught for the first time were banded with a USGS numbered metal band as well as a unique combination of three plastic color bands to allow further identification from a distance. For each capture, birds were first weighed then had the length of their beak, head plus beak, tarsus, tail and wing measured (Pyle, 1997). Following these measurements and depending on capture success, up to four birds per day were brought to the field station for metabolic measurement. All bird manipulations were approved by the animal care committee of the Université du Québec à Rimouski (CPA-37-09-68) and have been conducted under scientific and banding permits from Environment Canada - Canadian Wildlife Service (Permit Number: 10704H).

1.4.2 Respirometry

Once at the field station, birds were maintained in separate cages (39 cm x 43 cm x 31 cm) supplied with food (sunflower seed) and water *ad libitum* until measurements were made. Cages were kept in a room receiving natural light through a window and maintained quiet to avoid disturbance. At around 13:00, we began Msum trials by measuring two birds in parallel using FoxBox oxygen analyzers (Sable Systems, Las Vegas, NV, USA). This was followed by a second trial on the remaining two birds, which began before 15:00. Briefly, birds were first weighed (± 0.1 g) and body temperature was measured with a thermocouple reader (Omega model HH-25KC, NIST-traceable, Omega, Montréal, Qc, Canada) using a copper-constantan thermocouple inserted into the cloacae approximately 10 mm deep. Then, birds were put in a stainless steel metabolic chamber fitted with a perch and were exposed to helox gas (21% oxygen, 79% helium) using an average flow rate of 1109 $ml.min^{-1}$ controlled by mass flow valves (Sierra Instruments, Side-Trak® Model 840, Monterey, CA, USA). We recorded oxygen consumption of each bird using a sliding cold exposure protocol (Swanson *et al.*, 1996) with a decrease in ambient temperature of 3°C every 20 minutes, starting at 0°C in winter and at 6°C in summer. We ended the trials when birds became hypothermic, which was easily identifiable in real time as a steady decline in oxygen consumption for several minutes. Body temperature was measured again immediately after taking birds out of their chambers. We assumed a bird had reached its Msum when body temperature after a trial was ≤ 38°C (Cooper & Gessaman, 2005) (mean body temperature after Msum measurement = 33.6 ± 0.2°C). Data from individuals showing a body temperature above this threshold were discarded. Birds were weighed again after measurements and the average body mass was used for the Msum analysis. Birds were then brought back to their cage with food and water *ad libitum* until BMR measurement starting at 19:00.

Each day, all four birds had their BMR measured simultaneously overnight (from 19:00 to 06:00). Individuals were maintained at 30°C throughout the trial (within the thermoneutral zone for this species, Rising & Hudson, 1974) and received a constant flow of air. Birds were weighed before and after measurements and average mass was used in BMR analyses.

Oxygen analyzers were adjusted each day to 20.95% using CO_2-free dry air and mass flow valves were carefully calibrated for air and helox using a bubble-O-meter (Dublin, OH, USA)

once per winter. Metabolic rate calculations were done with ExpeData software, v1.2.6 (Sable Systems, Las Vegas, NV, USA). Msum and BMR calculations were based respectively on the highest and lowest averaged 10 minutes of oxygen consumption per measurement sequence according to Lighton's equation 10.1 (2008). The instantaneous measurement technique (Bartholomew *et al.*, 1981) was used for Msum while BMR was calculated using the steady state approach. The duration of BMR trials (around 11 hours) insured that birds were post-absorptive at time of BMR measurement (which was obtained after 6 h 40 min ± 8 min of recording on average). Since wintering birds use mostly lipids as substrate for shivering (Dawson *et al.*, 1983; Swanson, 2010; Vaillancourt *et al.*, 2005), we estimated energy consumption using a constant equivalent of 19.8 $kJ.L^{-1}$ O_2 and converted to Watts (Gessaman & Nagy, 1988). After BMR measurements, birds were put back in their cage with access to food and water until release at capture site around 2 hours later.

1.4.3 Sexing individuals

130 individuals (56 females and 74 males) caught for this study were sexed by PCR (Griffiths *et al.*, 1998) or dissection. We determined sex of the remaining 98 birds using their morphometric data in a discriminant analysis (Desrochers, 1990). Overall, 85 individuals were identified as females, 107 as males and 36 birds remained undetermined.

1.4.4 Statistical analysis

1.4.4.1 Inter-seasonal variation

We first studied inter-seasonal variation in body mass and metabolic performance. To do so, we used a linear mixed effect model (LME) to test for effects of "year", "season" (winter or summer), "sex" (male, female or undetermined) and interaction term "year*season", using bird ID as random parameter, on whole BMR, Msum and ME. We also included the variable "relative time of capture" (time since sunrise / day length, hereafter "time of capture") for body mass analyses. We then used the same model including body mass as a covariate to analyse variations in mass-independent metabolic performance.

1.4.4.2 Intra-seasonal variation

To study intra-seasonal variation (*i.e.* within winter) in parameters of metabolic performance, we used the same model but this time testing for the effects of "year", "month", "sex" and the interaction term "year*month", again using bird ID as random parameter. "Time of capture" was also considered when analysing variations in body mass. To study effects on mass-independent variables we repeated the analyses including body mass as a covariate.

We saved residuals from those LMEs to study relationships between whole and mass-independent winter BMR and Msum.

Variables were removed from models when non-significant and results from final models are presented. In cases where the interaction term year*month was significant, we ran separate analyses by year. We used Tukey tests to investigate differences between months and between sexes. Analyses on metabolic parameters showed the same final patterns whether or not body mass was included as a covariate. We therefore present results for mass-independent BMR, Msum and ME. Data for these variables without mass corrections are available in the Annexes (tables A1 and A2). In all cases, residuals were tested for normality using the one sample Kolmogorov-Smirnov test. Population data are presented as least square means ± s.e.m. and intra-individual data are shown as predicted values (original values corrected for the effects that were found significant at the population level).

1.5 Results

1.5.1 Temperature

During the first winter of the study (2009-2010), chickadees experienced mean daily temperatures below 0°C from December to March (table 1.1). The coldest average temperature was recorded in December and the lowest minimal temperature was measured in February. During the second year (2010-2011), mean temperatures fell below 0°C from November to March and reached their coldest mean ambient and minimal values in January (table 1.1). However, the following months remained relatively cold as minimal temperatures changed by

less than one degree in the next two months. Overall, the second winter of the study was colder than the first and had more months with minimal temperatures below -25°C.

Table 1.1 Monthly minimal, mean and maximal temperatures (°C) recorded by weather station within the study area.

	2009/2010			2010/2011		
Months	T_{Min}	T_{Mean}	T_{Max}	T_{Min}	T_{Mean}	T_{Max}
October	-6.8	3.1	13.5	-3.2	5.7	19.3
November	-7.3	2.2	13.5	-12.6	-1.2	13.5
December	-20.3	-7.3	2.1	-16.4	-3.6	11.7
January	-21.1	-6.3	8.3	-26.8	-11.4	2.0
February	-24.7	-5.7	6.5	-26.3	-11.2	3.1
March	-15.8	-1.9	9.6	-26.8	-5.4	8.4
August	5.6	18.2	30.7	-	-	-

(Data for August 2011 not available)

1.5.2 Inter-seasonal variation in body mass and metabolic performance

Peak values in average metabolic performance were recorded in February for both years (see below). We therefore calculated inter-seasonal variation in body mass and metabolic parameters between peak of winter and summer using values measured in February and August.

Average body mass did not vary between years or seasons but positively varied with time of capture ($F_{1, 70} = 11.2$, $p < 0.01$). Males were also on average 10.5% heavier than females (sex: $F_{2,112} = 59.8$, $p < 0.0001$, males: 12.06 ± 0.07 g; females: 10.91 ± 0.08 g, undetermined 11.31 ± 0.10 g, Tukey: $p < 0.0001$). BMR, Msum and ME were all influenced by body mass (BMR: $F_{1, 112} = 32.5$, $p < 0.0001$; Msum: $F_{1,109} = 29.6$, $p < 0.0001$, ME: $F_{1, 98} = 5.0$, $p < 0.05$). Mass-independent BMR was 5.9% higher in winter relative to summer (season: $F_{1, 116} = 16.5$, $p < 0.0001$, figure 1.1A) while average values for mass-independent Msum were 34.2% higher at the peak of winter relative to August (season: $F_{1, 115} = 135.5$, $p < 0.0001$). However, this latter effect depended on the year (year*season: $F_{1, 117} = 7.2$, $p < 0.01$). Mass-independent Msum was 13.4% higher in the first winter relative to the second, which led to a Msum being 41.9% higher than in summer during the first year compared to a 26.4% seasonal difference during the second year (Tukey: $p < 0.0001$ in all cases) (figure 1.1B). Mass-independent ME was 25.0% higher in winter (6.2 x BMR) than in summer (5.0 x BMR, season: $F_{1, 111} = 56.9$, $p < 0.0001$) and this effect was also dependent on the year (year*season: $F_{1, 109} = 5.2$, $p < 0.05$). Mass-independent

ME was 11.4% higher in the first winter than in the second with a ME 32.7% higher relative to summer in the first year compared to a 17.4% difference between winter and summer during the second year (Tukey: $p < 0.0001$ in all cases) (figure 1.1C).

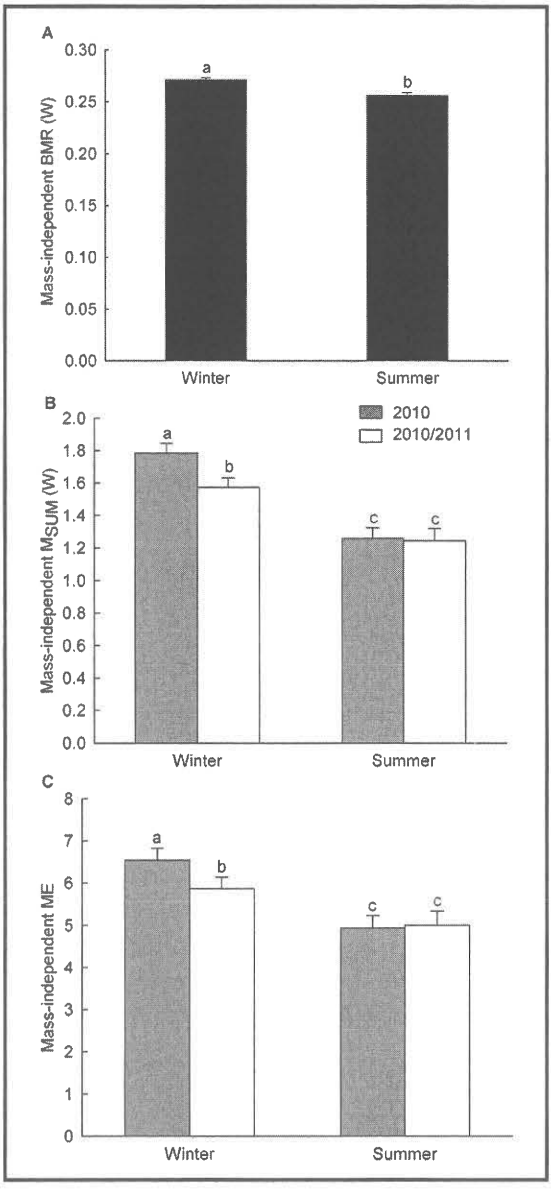

Figure 1.1 Inter-seasonal variation of mass-independent BMR, Msum and ME. Data are least square means for BMR (A), Msum (B) and ME (C) controlling for year, season, sex and body mass with bird ID as random parameter. Different letters indicate significant difference between seasons.

1.5.3 Intra-seasonal variation of body mass and metabolic performance

As for the inter-seasonal analyses, we found no year effect on average body mass in wintering Black-capped chickadees. However, body mass varied over time within winter (month: $F_{6, 212} = 8.3$, $p < 0.0001$) increasing by 4.0% between October and December (Tukey: $p < 0.01$) and remained constant until August (figure 1.2, Tukey: $p = 0.07$). Mass also differed between sexes ($F_{2, 218} = 146.8$, $p < 0.0001$) with males being on average 11.5% heavier than females (males: 12.00 ± 0.05 g; females: 10.76 ± 0.06 g, undetermined 11.18 ± 0.07 g, Tukey: $p < 0.0001$) and, as these birds are fattening up on a daily basis (Lehikoinen, 1987; Mandin & Vézina, 2012), body mass was positively affected by time of capture ($F_{1, 225} = 57.1$, $p < 0.0001$).

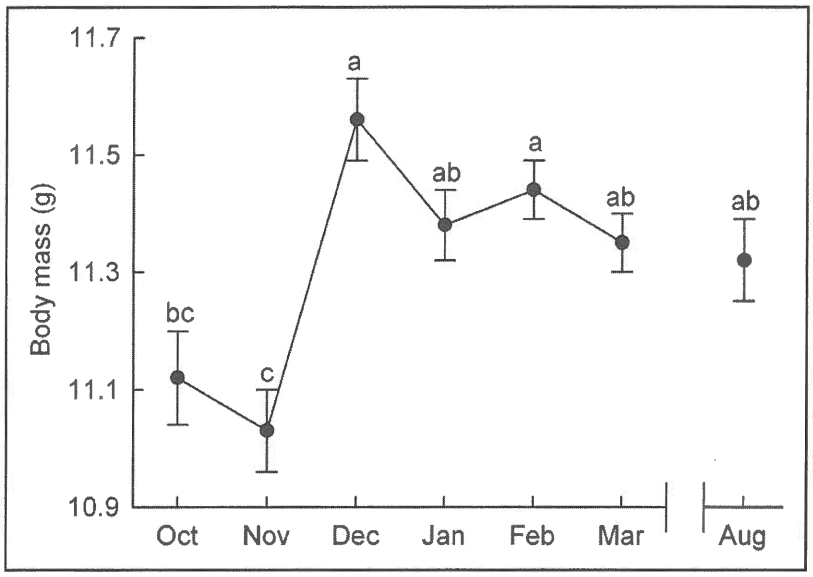

Figure 1.2 Intra-seasonal changes in body mass. Data are least square means of body mass controlling for year, month, time of capture, sex with bird ID as random parameter. Different letters represent significant difference between months.

Mass-independent BMR (body mass: $F_{1, 218} = 83.1.2$, $p < 0.0001$) did not change between years but varied within winter (month: $F_{6,321} = 6.1$, $p < 0.0001$, figure 1.3A). BMR progressively increased by 5.9% between October and February (Tukey: $p < 0.05$) and then decreased by 5.5% between February and March (Tukey: $p < 0.0001$).

As for BMR, mass-independent Msum (body mass: $F_{1, 204} = 97.6$, $p < 0.0001$) did not differ between years but varied within winter (month: $F_{6, 270} = 37.1$, $p < 0.0001$). Average Msum increased by 25.0% between October and February and weakly decreased by 5.2% between February and March. However, the month effect also depended on the year (year*month: $F_{3, 266} = 10.0$, $p < 0.0001$). Separated analyses by year showed that the Msum peak observed in February was only apparent in 2010, where it reached a value 31.9% higher than our intra-seasonal reference point in October, before declining by 13.3% between February and March (Tukey: $p < 0.01$). During the second year, mass-independent Msum increased steadily throughout winter to reach a peak in March with a value +20.7% higher than that measured in October (Tukey: $p < 0.0001$) (figure 1.3B).

Mass-independent winter metabolic expansibility (body mass: $F_{1, 156} = 8.2$, $p < 0.01$) varied with month ($F_{6, 253} = 18.3$, $p < 0.0001$), with average mass-independent ME increasing by 19.8% between October and March. However, the month effect was also dependent on the year (year*month: $F_{3, 253} = 4.9$, $p < 0.01$). Separated analyses revealed that during the first year, mass-independent ME reached a peak (6.5 x BMR) in February (+27.0% relative to October 2011) before decreasing non-significantly in March (-2.6%, Tukey: $p = 0.8$). During the second year, mass-independent ME reached its highest value (6.2 x BMR) in March (+19.6% relative to October, Tukey: $p < 0.0001$) (figure 1.3C).

For all metabolic parameters, visual inspection of predicted values for recaptured individuals showed a high level of variability between birds but consistency in their position relative to others (*i.e.* there were "high" and "low" BMR/Msum individuals). Individual patterns were comparable to that observed at the population level (figure 1.3).

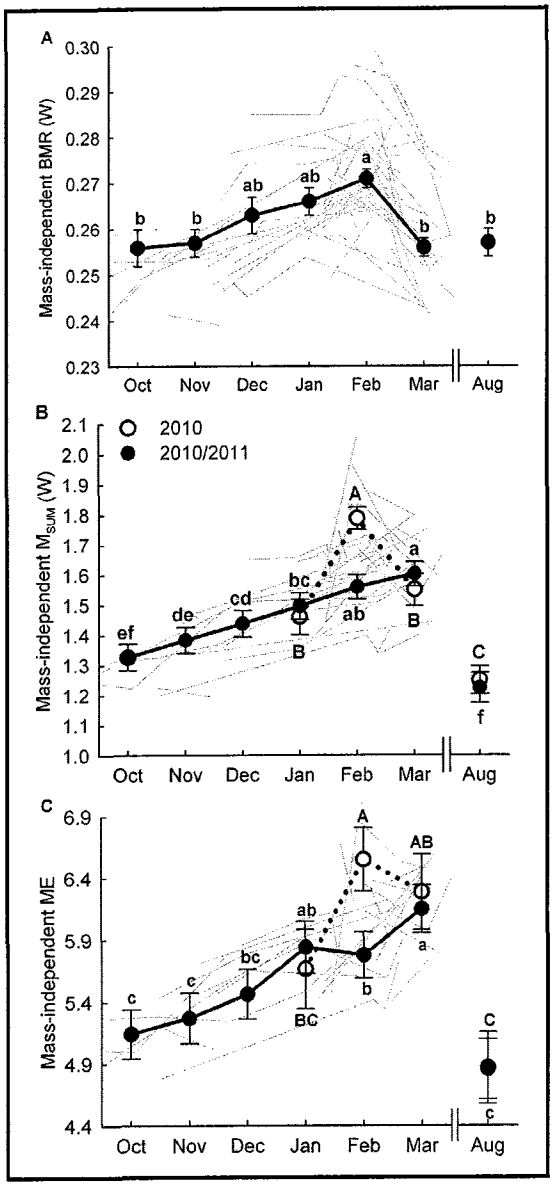

Figure 1.3 Monthly variation at the population and individual levels in mass-independent BMR, Msum and ME. Population data (black line and dots) are least square means for BMR (A), Msum (B) and ME (C) controlling for year, month, sex, body mass and bird ID as random parameter. In B and C - dotted line, open dots and uppercase: 1st year; solid line, black dots and lowercase: 2nd year. Different letters represent significant difference between months within a year. Predicted values calculated from LMEs (see text for details) are used to visualize data from individuals captured more than once (grey lines).

1.5.4 Relationship between BMR and Msum

Linear regression between residuals of whole BMR and whole Msum extracted from linear mixed effect models resulted in a significant but weak positive relationship between these parameters ($n = 269$; $r^2_{adj} = 0.04$; $p < 0.001$). The relationship was not significant when using residuals from models controlling for body mass ($p = 0.4$).

1.6 Discussion

This study is the first to document, with an extensive dataset, intra-seasonal and intra-individual patterns of change (*e.g.* reaction norm, McKechnie, 2008) in metabolic performance of free-living birds wintering at northern latitudes. As expected, both BMR and Msum were higher in winter relative to summer and increased during the coldest months of winter. However, average BMR and average Msum followed dissimilar paths with BMR declining to summer level in the spring while Msum tended to remain high, resulting in the highest metabolic expansibility being recorded in March. Variations in metabolic performance observed at the population level reflected that observed within individuals.

1.6.1 Inter-seasonal variation in metabolic performance

Mass-independent basal metabolic rate, interpreted here as the energy expenditure of physiological systems remaining active in a resting bird, peaked at 0.27 W in wintering Black-capped chickadees. This is comparable to values measured in wintering chickadees from Ohio (0.26 W) and Wisconsin (0.27W) (Olson *et al.*, 2010) but lower than the ones measured in birds spending their winter in New York (0.29) (Chaplin, 1974), South Dakota (0.30 W) (Olson *et al.*, 2010) and Alaska (0.42 W) (Grossman & West, 1977). It is therefore not surprising to find that the 6% seasonal increase in mass-independent BMR observed here is much lower than the +14% found by Cooper and Swanson in birds from South Dakota (Cooper & Swanson, 1994). This seasonal variation is, however, in the relatively large range of seasonal changes observed in other temperate free-living resident passerines (from -4.3% in *Carpodacus mexicanus*, O'Connor, 1995 to +36.3% in *Passer montanus*, Zheng *et al.*, 2008). Assuming the seasonal elevation in

BMR reflects an increase in maintenance costs (Swanson, 1991a; Zheng *et al.*, 2008), it therefore appears that Black-capped chickadees from our study site only face a moderate rise in maintenance energy demand in association with seasonal cold acclimatization relative to other populations. If temperature is one of the drivers of winter metabolic performance (Broggi *et al.*, 2007; Swanson & Olmstead, 1999), it is likely that differences between populations reflect physiological responses to local conditions.

As for BMR, the seasonal increase in Msum (+34%) was in the range of previously reported observations. Seasonal changes in mass-independent Msum range from +16.2% in *Sitta carolinensis* (Liknes & Swanson, 1996; O'Connor, 1995) to +42.4% in *Picoides pubescens* (Liknes & Swanson, 1996), with Black-capped chickadees from South Dakota showing a 26.3% increase in winter mass-independent Msum relative to summer (Cooper & Swanson, 1994).

1.6.2 Intra-seasonal variation in metabolic performance and the uncoupling of BMR and Msum

Temperature has been suggested as one of the drivers of winter metabolic performance (Broggi *et al.*, 2007; McKechnie & Swanson, 2010; Olson *et al.*, 2010; Swanson & Olmstead, 1999) and this led us to predict a gradual increase in BMR and Msum during winter where a peak would be observed during the coldest months of the season. Seasonal variations in BMR were of lower amplitude than expected but changes in average values were consistent with our prediction. Although significant differences were only clear when comparing February with October, November and March, BMR clearly tended to be higher during December, January and February, the coldest months of winter (both years combined, table 1.1). However, BMR declined rapidly in March, when ambient temperatures were still relatively cold (-16°C and -27°C mean minimum temperature in 2010 and 2011 respectively), which suggests that temperature may not be the sole driver of winter BMR phenotype in chickadees.

In contrast, intra-seasonal changes in average Msum differed between years. During the first winter, Msum peaked in February and declined in March while in the second year Msum increased until the end of our measurements in March. Msum variations were therefore consistent with our predictions for the first year, where February was the coldest month based on minimal temperature, but were counterintuitive for the second year where no decline in Msum

were observed. It must be noted, however, that cold energy-demanding temperatures lasted much longer during the second winter since, although daily temperatures were already warming by March, minimal temperatures were as cold in March as in January. It is therefore likely that birds maintained their thermogenic capacity to its maximal level as long as days with very cold temperature prevailed. As metabolic expansibility is the ratio of Msum on BMR, and given the differences in range of intra-seasonal changes in BMR and Msum, variations in ME were inevitably affected by changes in Msum. It was therefore of no surprise to find similar variation when comparing ME and Msum.

The contrast in BMR and Msum patterns goes in hand with the hypothesis that these components of metabolic performance respond to different sets of environmental constraints (McKechnie & Swanson, 2010; Swanson *et al.*, 2012; Vézina *et al.*, 2006). Comparing intra-seasonal patterns of change in BMR and Msum relative to summer values also suggests a certain level of independence between these parameters. Although it was moderate, the increase in mass-independent BMR between seasons (+5.9% comparing August and February) was the same as that detected between October and February (+5.9%). In fact, the increase in BMR started after November, when birds began to face minimal temperatures below -10°C (table 1.1), and remained relatively steady until February (figure 1.4A). In contrast, the inter-seasonal change in mass-independent Msum (+34.2% between August and February) was higher than that measured within season (+25.0% between October and February). In fact, Msum had already achieved 21.8% of its inter-seasonal increase when we began our measurements in October (figure 1.4B) meaning that, although the largest change in Msum appeared between January and February (+45.4% of total inter-seasonal increase, figure 1.4B), this parameter began to change well before the beginning of sub-zero mean ambient temperatures (table 1.1). These results therefore suggest that flexible adjustments in thermogenic capacity appear relatively early in autumn (before October) while physiological components reflected in BMR only begin to change later (from November).

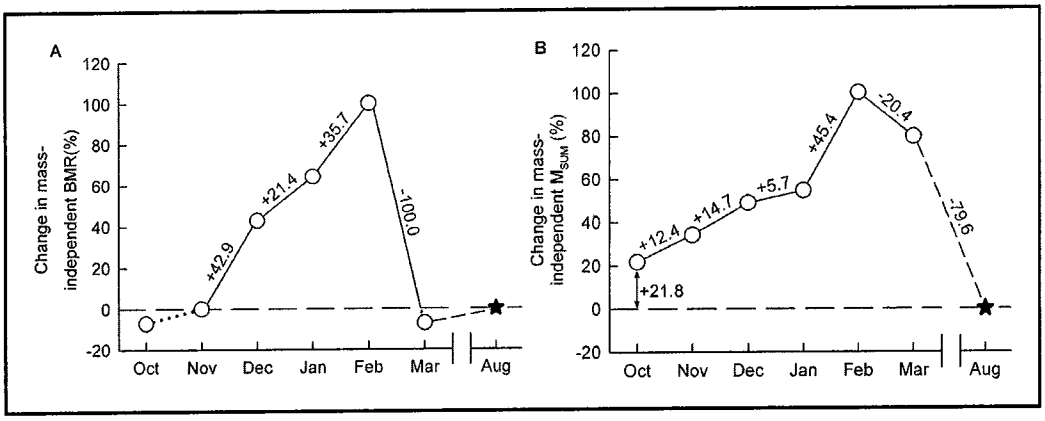

Figure 1.4 Visual representation of the monthly winter increment in mass-independent BMR (A) and Msum (B) relative to summer values. Data measured in August are used as the summer phenotype reference point (August = 0%, black star, dashed line) while maximal levels of BMR and Msum recorded in winter are considered representative of the "peak" winter phenotype (February = 100%). Monthly changes in metabolic performance (values above lines) are presented in percent of total inter-seasonal difference. Dotted lines represent BMR variations below the summer reference point.

Why would BMR begin to increase later in autumn and decline earlier in the spring than Msum? The reasons for the uncoupling of these variables are not clear. However, if one considers Msum as a variable mainly influenced by the size of shivering muscles (Cooper, 2002; O'Connor, 1995; Saarela & Hohtola, 2003; Vézina *et al.*, 2007) and BMR as reflecting changes in size and activity of the main digestive organs in response to cold acclimatization (Liu & Li, 2006; Maldonado *et al.*, 2009; Zheng *et al.*, 2008) then it is likely that those metabolic parameters reflect different sets of physiological constraints acting relatively independently, both being associated with winter (Vézina *et al.*, 2010). Pectoral muscles would start changing early in the season in association with the appearance of cold ambient temperatures (note that sub-zero minimal temperatures were already recorded in October) whereas winter BMR variations would likely reflect changes in diet and in the amounts of food consumed (to sustain an increasing daily energy expenditure, DEE). Chickadees feed mainly on insects during summer but eat up to 50% vegetal matter during winter (Smith, 1993) while food supplementation by feeders may represent only up to 21% of their daily energy intake (Brittingham & Temple, 1992). Snow typically starts to fall in November at our field site while insects are already visible by March. Therefore, it could be that the combination of a winter increase in DEE and changes in natural diet

digestibility and energy content leads to a restructuring of the digestive apparatus, and in turn changes in BMR, appearing independently from those observed in Msum. The proximal effect of cold ambient temperature on parameters of metabolic performance (Broggi *et al.*, 2007; Olson *et al.*, 2010; Swanson & Olmstead, 1999) would therefore be much more influential for Msum than for BMR. Experimental research is needed to test this "metabolic uncoupling" hypothesis and to determine what biotic and/or abiotic factors trigger seasonal changes in parameters of metabolic performance.

Given the seasonal mismatch in variations of BMR and Msum, it is therefore of no surprise to find a lack of significant correlation between these parameters when controlling for body mass in regression analyses. Independence of BMR and Msum has also been observed by others (Swanson, 2010; Swanson *et al.*, 2012; Vézina *et al.*, 2006; Wiersma *et al.*, 2007) but findings remains conflicting (Dutenhoffer & Swanson, 1996; Lewden *et al.*, 2012). Experimental manipulations of BMR and Msum, for example by combining diet and temperature treatments, should therefore be conducted to confirm findings.

1.6.3 Intra-individual variation in winter metabolic performance

Studies on seasonal variation of avian metabolic performance are typically conducted at the population level (Cooper, 2000; Liknes & Swanson, 2011b; O'Connor, 1995; Zheng *et al.*, 2008) and, although it is rarely stated, they generally assume that population patterns are reflective of those observable within individuals. As far as we know, this is the first study to document with an extensive dataset seasonal variation of metabolism at both the population and individual levels in a resident bird species. Our findings support the common assumption; patterns observed at the population level reflected intra-individual variation in body mass, mass-independent BMR, Msum and ME (figure 1.3) and are therefore representative of average individual phenotypic flexibility.

1.6.4 Is metabolic expansibility a meaningful variable?

In this study, we considered metabolic expansibility as an indicator of the capacity of an organism to produce heat (because it was based on Msum) for a given size of metabolic machinery (which would be reflected by BMR) (Arens & Cooper, 2005; Cooper & Swanson, 1994; Hinds *et al.*, 1993; Swanson, 2010; Swanson *et al.*, 2012). However, the lack of correlation between BMR and Msum, as observed by others (Swanson, 2010; Swanson *et al.*, 2012; Vézina *et al.*, 2006; Wiersma *et al.*, 2007), as well as the temporal mismatch in these parameters during seasonal acclimatization suggest a lack of a functional relationship between BMR and Msum. Although BMR includes the resting energy consumption of physiolgical components involved in active thermogenesis (*i.e.* muscles), evidences suggest that, in the context of cold acclimatization, its variations are mainly influenced by activity and size of organ systems involved in energy acquisition and digestion (Liknes & Swanson, 2011b; Liu & Li, 2006; Zheng *et al.*, 2008) while Msum would mostly reflect active energy consumption of shivering muscles (Cooper, 2002; Saarela & Hohtola, 2003; Vézina *et al.*, 2007). It therefore becomes apparent to us that metabolic expansibility, as we defined it earlier, may lead to misleading conclusions about machinery adjustments. We therefore suggest caution with the interpretation of this variable and recommend interpreting variations in BMR and Msum separately to infer on animals metabolic capacity.

Acknowledgments

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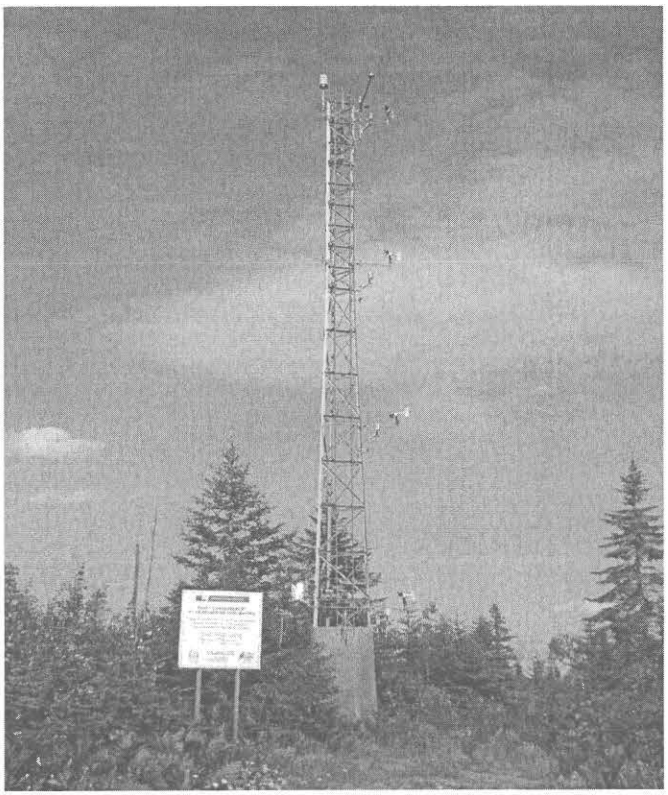
CHAPITRE II

Normes de réaction en conditions naturelles : comment les petits endothermes ajustent-ils leur performance métabolique aux variations météorologiques ?



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Reaction norms in natural conditions: how does metabolic performance respond to weather variations in a small endotherm facing cold wintering environments?

Magali Petit, François Vézina

Département de biologie, chimie et géographie, Université du Québec à Rimouski, Rimouski (QC), Canada. Groupe de recherche sur les environnements nordiques BORÉAS, Rimouski, (QC), Canada. Centre d'Études Nordiques, Québec (QC), Canada. Centre de la Science de la Biodiversité du Québec, Montréal (QC), Canada.

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Contributions - Conception de l'expérience: MP, FV ; Réalisation de l'expérience: MP; Analyse des données: MP ; Financement: FV ; Rédaction : MP, FV.

2.1 Résumé

Les normes de réaction reflètent la capacité des organismes à ajuster leur phénotype à leur habitat et permettent de mettre en évidence les conditions environnementales auxquelles les animaux atteignent leurs limites physiologiques. Cependant, les normes de réaction de paramètres physiologiques sont rarement étudiées chez les endothermes en conditions naturelles. Les mésanges à tête noire (*Poecile atricapillus*) augmentent leur performance métabolique durant l'acclimatation hivernale et sont donc un bon modèle pour étudier les normes de réaction de paramètres physiologiques en milieu naturel. Nous avons mesuré le métabolisme de base (BMR) et le métabolisme maximal induit par le froid (Msum) chez des mésanges capturées plusieurs fois au cours de l'année afin de caractériser, pour la première fois chez un endotherme en liberté, les normes de réaction de ces paramètres physiologiques le long d'un gradient de conditions météorologiques. Nous avons montré que le BMR différait entre les individus et était faiblement et négativement relié à la température minimale. Quant au Msum, il s'ajustait avec la température minimale selon une courbe sigmoïde, augmentant linéairement entre 24°C et -10°C, et il variait avec l'humidité absolue selon une courbe en U. Ces résultats impliquent que les échanges de chaleur avec l'environnement auraient des effets minimes sur les coûts de maintenance, qui seraient surtout individu-dépendant, alors qu'au contraire les pertes de chaleur corporelles influenceraient la capacité thermogénique. Ceci suggère aussi que le BMR et le Msum répondraient à des contraintes différentes et probablement indépendantes.

Mots-clefs: ajustements physiologiques; flexibilité phénotypique; métabolisme; acclimatation; oiseau; endotherme.

2.2 Abstract

Reaction norms reflect an organisms' capacity to adjust its phenotype to the environment and allow for identifying trait values associated with physiological limits. However, reaction norms of physiological parameters are mostly unknown for endotherms living in natural conditions. Black-capped chickadees (*Poecile atricapillus*) increase their metabolic performance during winter acclimatization and are thus good model to measure reaction norms in the wild. We repeatedly measured basal (BMR) and summit (Msum) metabolism in chickadees to characterize, for the first time in a free-living endotherm, reaction norms of these parameters across the natural range of weather variation. BMR varied between individuals and was weakly and negatively related to minimal temperature. Msum varied with minimal temperature following a sigmoid curve, increasing linearly between 24°C and -10°C, and changed with absolute humidity following a U-shape relationship. These results suggest that thermal exchanges with the environment have minimal effects on maintenance costs, which may be individual-dependent, while thermogenic capacity is responding to body heat loss. Our results suggest also that BMR and Msum respond to different and likely independent constraints.

Keywords: physiological adjustments; phenotypic flexibility; metabolic rate; acclimatization; bird; endotherm; BMR; Msum.

2.3 Introduction

Phenotypic flexibility is the ability of a fully-developed organism to rapidly and reversibly adjust its phenotype to track short-term environmental changes (Piersma & Drent, 2003). Because it allows for individuals to match their physiology with the requirements of their surroundings, phenotypic flexibility should improve survival and thus influence fitness (Brommer, 2013; Wilson & Franklin, 2002).

The capacity to adjust phenotypic traits to changes in the environment is studied through reaction norms, which describes the flexibility of a trait across an environmental gradient (figure 2.1) (McKechnie, 2008; Nussey *et al.*, 2007; Schlichting & Pigliucci, 1998). According to Nussey *et al.* (2007), Brommer (2013) and Mc Kechnie (2008), reaction norms can be characterized by four parameters. The *elevation* is the mean trait expression (*i.e.* the intercept), the *slope*, which represents phenotypic flexibility, measures the change in trait value for a given change in an environmental parameter, the *amplitude* is the difference between minimal and maximal trait values and the *shape* (*e.g.* linear, sigmoid) informs on the limits of adjustment in a trait over a given range of change in the environment.

Reaction norms can be studied both at individual (figures 2.1a and b) and population levels (figure 2.1c) (Brommer, 2013; Charmantier *et al.*, 2008). In a first scenario (figure 2.1a), individuals with different elevations and slopes show different phenotypes and phenotypic responses to changes in their environment. If phenotypic flexibility is under natural selection (Scheiner & Lyman, 1991), environmental stochasticity should promote survival of the most flexible individuals, leading to microevolution at the population level (Nussey *et al.*, 2007). In a second scenario (figure 2.1b), animals with different elevations but similar slopes express different phenotypes but have the same flexibility. Individuals with the highest elevation would consistently remain high relative to others (*i.e.* repeatable trait) and, assuming that a high elevation in that trait increases fitness, they would perform better than their counterparts (Wilson & Nussey, 2010). In a third scenario (figure 2.1c), individuals express similar elevations and slopes resulting in individuals with comparable phenotypes and phenotypic flexibilities. In this situation, individual phenotypic flexibility would reflect the adjustment capacity of the population (Nussey *et al.*, 2007).

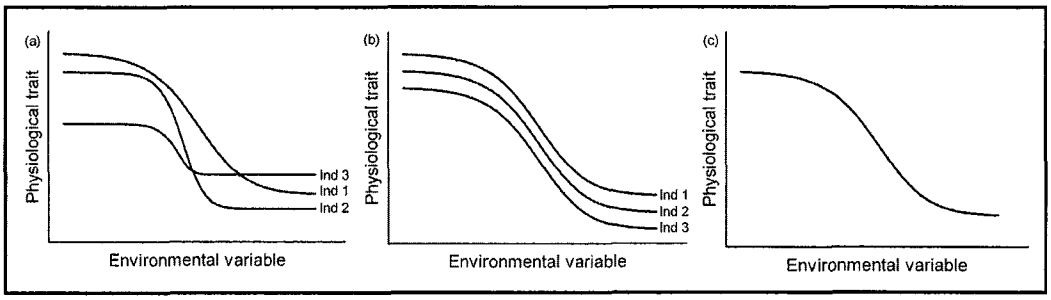

Figure 2.1 Reaction norms according to three scenarios. In (a) individuals express different elevations and slopes, in (b) individuals express different elevations but similar slopes and in (c) individuals express the same elevations and slopes, which reflect the population reaction norm.

Animals facing highly variable environments, such as those wintering at high latitudes, offer a great opportunity for studying phenotypic adjustments in natural conditions. Indeed, winter is a challenging period for non-hibernating endotherms living at northern latitudes (Chappell, 1980; Cooper, 2000) as they have to face low food availability (Swanson, 2010) at a time where high energy expenditure is required for thermoregulation (Liknes & Swanson, 1996). Winter metabolic phenotypes, reflecting individual rates of energy use, are commonly studied to understand individual performance and are measured through variables such as basal and summit metabolic rates (BMR and Msum, respectively). BMR is interpreted as the minimal maintenance energy cost of an animal and is thought to reflect metabolic activity of resting muscles and internal organs (Chappell *et al.*, 1999; Petit & Vézina, 2014a) while Msum is a measure of the cold-induced maximal heat production that is thought to reflect maximal shivering capacity of skeletal muscles (Petit & Vézina, 2014a; Swanson *et al.*, 2013). In small endotherms, both these parameters are typically elevated in winter relative to summer (Cooper & Swanson, 1994; Zhao *et al.*, 2010).

Adjustments in traits, such as metabolism, over a continuum of environmental variation are commonly investigated in ectotherms (Angilletta, 2009). However, only few studies are available for endotherms, with the majority focussing solely on the effects of winter ambient temperature (Broggi *et al.*, 2007; Jackson *et al.*, 2001; Swanson & Olmstead, 1999). Studies on acclimatization typically report differences between seasonal extremes with metabolic rates being higher in winter than in summer (Liknes & Swanson, 1996; Zhao *et al.*, 2010),

although observations for periods shorter than 6 months are also available (Bozinovic *et al.*, 2007; Liknes & Swanson, 2011b; Zhao *et al.*, 2010; Zheng *et al.*, 2008). Similarly, experimental studies investigating metabolic adjustments to thermal variations in endotherms typically use discrete changes (Maggini & Bairlein, 2013; Williams & Tieleman, 2000) rather than a continuous gradient of temperature. These approaches therefore limit conclusions to stable physiological states and provide little information on the dynamics of change in physiological parameters.

McKechnie (2008) suggested that flexibility of metabolic rate could be limited by physiological or morphological constraints, which should be observable in reaction norms including a linear part comprised between an upper and a lower plateau (*i.e.* sigmoid shape, figure 2.1). Studies on winter metabolic adjustments did highlight linear relationships between temperature and both BMR and Msum but did not test for non-linear effects (Jackson *et al.*, 2001; Swanson & Olmstead, 1999 but see Broggi *et al.*, 2007). Knowledge on the capacity of animals to respond to short-term environmental variability and on thermal thresholds at which endotherms could reach minimal and maximal metabolic values therefore remains limited.

Here, we used the Black-capped chickadee, a small (9-14g) North-American non-migratory passerine, as our model species to investigate adjustments of BMR and Msum to natural variations in weather parameters. Birds express higher metabolic rates than mammals of comparable size (Hulbert *et al.*, 2007) and, given their high surface/volume ratio, small species are highly sensitive to heat loss. This makes the chickadee a perfect model to investigate the effects of weather variability on metabolic flexibility. Chickadees also defend small territories during winter (Smith, 1991), which facilitates recaptures and allows for obtaining sequences of individual measurements in varying conditions. Although temperature undeniably affects avian metabolic rates (McKechnie & Swanson, 2010; Swanson & Olmstead, 1999), heat transfer also involves other parameters such as solar radiation, humidity and wind speed (Bakken *et al.*, 1991; Hayes & Gessaman, 1980; Robinson *et al.*, 1976; Walsberg & Wolf, 1995). We therefore considered an array of parameters rather than only the effect of ambient temperature. We expected that, in natural conditions, reaction norms would follow non-linear patterns over the seasonal range of weather variation, as

metabolic rates should be limited by physiological constraints (McKechnie, 2008). Using random regressions, we tested whether metabolic adjustments differed between individuals in elevation and slope (scenario 1), in elevation only (scenario 2) or neither (scenario 3). To the best of our knowledge, this is the first study to investigate reaction norm of BMR and Msum over natural weather gradients in a free-living population of endotherms.

2.4 Materials and methods

2.4.1 Capture and handling

This field study was conducted at the Forêt d'Enseignement et de Recherche Macpès, Québec, Canada (48°18' N, 68°31' W) from August 2010 to march 2011. Sixteen capture stations were distributed within the 2300 ha of the forest and were set up with feeders (Perky-Pet® 10" Sunflower Seed and Peanut Feeder) filled with black sunflower seeds. During capture sessions (between 08:00 and 13:00), feeders were removed and homemade potter traps (15 cm x 15 cm x 15 cm) baited with seeds were used to catch birds, which allowed us to handle individuals as soon as they were trapped. We caught 183 individuals and recaught 45 birds (25%), with an average rate of 1.3 ± 0.2 recapture *per* bird (table 2.1). The average duration between two captures was of 45 ± 2 days. Birds caught for the first time were banded with a USGS numbered metal band for identification. After capture, birds were weighed and measured following standardized protocols (length of beak, head plus beak, tarsus, tail and wing) (Petit *et al.*, 2013). Following these measurements, up to four birds per day were brought to the field station for metabolic measurements (see Petit *et al.*, 2013; Petit & Vézina, 2014a for other studies on the same population in which this dataset is also included, for example in multi-year analyses).

Table 2.1 Sample sizes presented relative to the number of capture per bird.

Number of capture/bird	Sample size
1	138
2	27
3	11
4	1
5	3
6	2
7	1

2.4.2 Respirometry

At the field station, birds were kept at room temperature in separate cages (39 cm x 43 cm x 31 cm) supplied with food (sunflower seed) and water *ad libitum* until metabolic rate measurements. Cages were kept in a quiet room receiving natural light. At around 13:00, we measured the Msum of two birds simultaneously using the instruments and protocol described by Petit *et al.* (2013). Measurement of the two remaining birds started before 15:00. Before Msum trials, birds were weighed (± 0.1 g) and body temperature (Tb) was measured with a thermocouple reader (Omega model HH-25KC, NIST-traceable, Omega, Montréal, QC, Canada) using a copper-constantan thermocouple inserted into the cloacae approximately 10 mm deep. Then, birds were put in metabolic chambers (effective volume = 1120 ml) fitted with a perch and a thermistor (Sable Systems UI2 AD converter, Sable Systems, Las Vegas, NV, USA) for chamber temperature measurements. We exposed the birds to helox gas (21% oxygen, 79% helium, average flow rate of 1109 $ml.min^{-1}$) and measured their oxygen consumption (FoxBox oxygen analyzers, Sable Systems, Las Vegas, NV, USA) using a sliding cold exposure protocol (Swanson *et al.*, 1996). This protocol involved a decrease in ambient temperature of 3°C every 20 minutes with trials starting at 6°C in summer, 3°C in fall and 0°C in winter. Trials ended when birds became hypothermic, which was detectable in real time as a steady decline in oxygen consumption for several minutes. At this time, birds were removed from metabolic chambers and their body mass (Mb) and Tb were measured again. We assumed a bird had reached its Msum when Tb after a trial was ≤ 38°C (mean Tb before Msum = 42.35 ± 0.04°C [pers.obs], thus average decline in Tb during Msum measurement > 4°C) (Cooper & Gessaman, 2005). Data from birds with Tb

above this threshold were discarded (n = 21) (mean Tb of hypothermic birds after Msum = 34.0 ± 0.2°C). Mb measured before and after trials were averaged and these values were used for Msum analyses. Birds were brought back to their cage with food and water *ad libitum* until BMR measurement commenced at night.

During BMR trials, up to four birds were measured simultaneously from 19:00 to 06:00. Measurements were done at 30°C (within the thermoneutral zone for this species, Chaplin, 1976) using a constant flow of air (average 470 $ml.min^{-1}$). As for Msum, birds were weighed before and after measurements and average Mb were used in BMR analyses.

Oxygen analyzers were adjusted each day to 20.95% O_2 using CO_2-free dry air. Mass flow valves (Sierra Instruments, Side-Trak® Model 840, Monterey, CA, USA) were calibrated for air and helox using a bubble-O-meter (Dublin, OH, USA). Metabolic rate calculations were done with ExpeData software, v1.2.6 (Sable Systems, Las Vegas, NV, USA). Using a 20 sec sampling interval for BMR and a 5 sec interval for Msum, BMR and Msum calculations were based respectively on the lowest and highest averaged 10 minutes of oxygen consumption per measurement sequence according to Lighton's equation 10.1 (Lighton, 2008). We applied the instantaneous measurement technique (Bartholomew *et al.*, 1981) for Msum calculations and a steady state approach for BMR. Duration of BMR trials (around 11 hours) insured that birds were post-absorptive at time of BMR measurement. Since birds use lipids as substrate during fasting and for shivering (Swanson, 2010), we estimated energy consumption using a constant equivalent of 19.8 $kJ.L^{-1}$ O_2 and converted to watts (Gessaman & Nagy, 1988). After BMR measurements, birds were put back in their cage with access to food and water until release on their capture site around 2 hours later.

2.4.3 Weather data

Body heat loss increases under cold conditions, we therefore considered weather parameters that would most likely affect energy expenditure of small birds during cold exposure. Hence, we used minimal ambient temperature (°C), maximal wind speed ($m.s^{-1}$), minimal absolute humidity ($g.m^{-3}$), minimal solar radiation ($W.cm^{-2}$) and minimal atmospheric pressure (kPa). We chose minimal values for temperature and maximal values for wind speed because a windy cold environment enhances heat loss. We used minimal absolute humidity since dry air facilitates

heat loss by evaporation and we employed minimal solar radiation because cloud cover reduces heat gain by radiation. Minimal barometric pressure was considered because storms, which could affect the birds' energy expenditure, are generally preceded by a decrease in atmospheric pressure.

Weather data were recorded during the study by three weather stations (station 1: 48°19'24" N, 68°31'23" W, altitude: 166 m; station 2: 48°17'50" N, 68°31'34" W, altitude: 176 m; station 3: 48°16'46" N, 68°33'05" W, altitude: 188 m) located within the Macpès forest. Each station included instruments at four heights (2 m, 8 m, 14 m and 20 m) that recorded wind speed each 2 minutes and ambient temperature, relative humidity (used to calculate absolute humidity), solar radiation and atmospheric pressure each 15 minutes. For each variable, values from all heights and stations were hourly averaged and these averages were used in our analysis. Unfortunately, equipment failure prevented us from recording accurate solar radiation data.

2.4.4 Sexing individuals

Of the 183 individual birds captured for this study, 99 (45 females and 54 males) were sexed by PCR analyses (n = 32) (Griffiths *et al.*, 1998) or by dissection after metabolic measurements (n = 67) (these birds were sacrificed for a study on organ size flexibility, Petit & Vézina, 2014a). These birds were then used to establish a discriminant function using morphometric data to determine the sex of the 84 remaining birds (21 females, 29 males, 34 undetermined) using discriminant analyses (Desrochers, 1990).

2.4.5 Statistical analysis

To analyse the relationship between metabolic performance and weather variations within the year, we first extracted residual BMR and residual Msum from ANCOVAs that analysed the effects of sex and body mass on whole BMR and Msum. Since minimal ambient temperature and absolute humidity were strongly correlated ($r > 0.90$), we could not include both parameters in models. We therefore used a polynomial regression to extract residual values for absolute humidity controlling for the effect of temperature and included this new variable in further analyses. To characterize the birds' capacity to adjust their BMR and Msum to weather

conditions, we investigated variations in residual BMR and residual Msum with random regressions (Brommer, 2013; Nussey *et al.*, 2007) including the four weather parameters (temperature, wind speed, residual absolute humidity and barometric pressure) as fixed effects (inter-correlations $r < 0.40$ in all cases). We also included the individual elevation (*i.e.* individual) and slopes (interaction between individual and each weather variable) as random effects to test for individual effects on metabolic performance adjustments (figure 2.1). Hence, for an individual i, the relationship between a phenotype P_i and an environmental variable E is defined as:

$$P_i = \left[\mu + \beta \times f\left(E, x\right)\right] + \left[m_i + b_i \times E\right] + \varepsilon$$

where the fixed effects μ and β are the population mean elevation and slope, the random effects m_i and b_i are the individual mean elevation and slope and the residual error is ε. f(E,x) is a polynomial function of E elevated to the order x, which allows for determining the shape of the relationship (if $x = 0$, no relationship; if $x = 1$, linear; if $x > 1$, non-linear).

We followed the top-down model selection strategy described by Zuur *et al.* (2009) to determine the best model explaining variations in residual BMR and residual Msum. The procedure goes as follow.

The first step was to determine the structure of the random effects. We used restricted maximum likelihood (REML) estimation to fit several models including the same fixed effects but different random effects. We then compared these models with likelihood ratio tests (LRT) following a chi-square distribution (χ^2) with one degree of freedom. Individual slopes were not significant for residual BMR ($p > 0.3$ in all cases) or residual Msum ($p > 0.5$ in all cases) and were therefore removed from models (*i.e.* scenario 1 was rejected for both metabolic parameters). When individual elevation was significant (*i.e.* data consistent with scenario 2), we calculated repeatability by dividing individual variance by the sum of individual and residual variances. Since multiple measurements on the same individual could potentially influence the data (Jacobs & McKechnie, 2014; Van de Pol & Verhulst, 2006), we used LRT to compare random regressions with autoregressive covariance structure to models with unstructured covariance matrix. For both residual BMR and residual Msum, there was no effect of the covariance structure and the correlation estimate was weak (BMR: $X^2 = 0.001$,

p = 0.97, phi = -0.005; Msum: X^2 = 1.27, p = 0.26, phi = 0.24). We therefore used the unstructured covariance matrix in further analyses.

In a second step, we determined the structure of the fixed effects by performing an automated selection based on the corrected Akaike Information Criterion (AICc) of models fitted by maximum likelihood estimation. This allowed us to identify the best models explaining residual BMR and residual Msum variation.

The third step was to use REML estimations to fit the best models and present the following results (full models are presented in Annexes, tables A3 and A.4). To visualize significant effects, we used second order local regressions (loess) with a smoothness parameter of 0.85 to fit curves to the data.

Analyses were performed in R version 3.0.3 (2014). Monthly raw values of BMR and Msum are provided in Annexes, tables A.5 and A.6.

2.5 Results

Both BMR and Msum were dependent on sex (BMR: $F_{2,239}$ = 4.6, $p < 0.05$; Msum: $F_{2,218} = 3.4$, $p < 0.05$) and body mass (BMR: $F_{1,239} = 131.4$, $p < 0.0001$; Msum: $F_{1,218} = 66.0$, $p < 0.0001$), with females expressing higher BMR than males (+3.5%, tukey: $p < 0.01$) and higher Msum than individuals of undetermined sex (+6.6%, tukey: $p < 0.05$). Consequently, in subsequent analyses we used the residuals of BMR and Msum, after controlling for sex and body mass.

The best model explaining variations in residual BMR included both minimal ambient temperature ($F_{1,239} = 15.8$, $p < 0.0001$) and individual elevation ($X^2 = 6.9$, $p < 0.01$, repeatability = 0.20). Therefore, BMR reaction norm was consistent with scenario 2. Individual BMR was repeatable and increased with a decline of ambient temperature (figure 2.2).

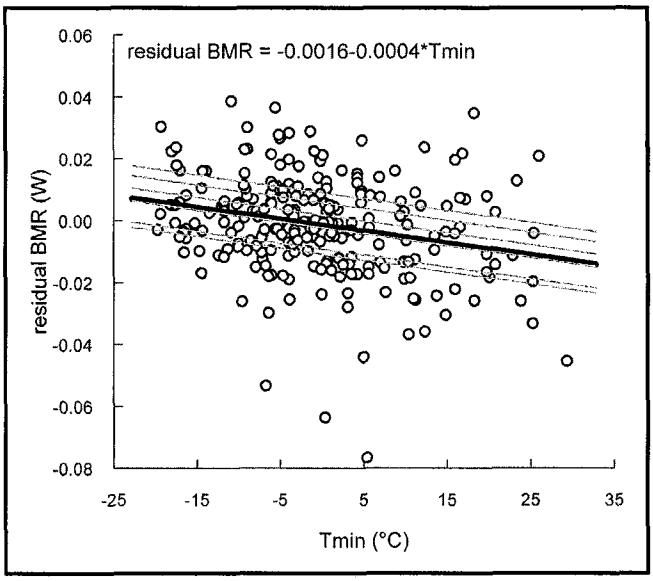

Figure 2.2 Relationship between mass and sex independent BMR and the natural range of minimal ambient temperature. Residual BMR were extracted from an ANCOVA controlling for body mass and sex. Population-average reaction norm is represented by the black line while grey lines represent reaction norms of individuals caught more than five times.

Residual Msum did not differ between individuals ($p = 0.1$) and its reaction norm was consistent with scenario 3. Indeed, residual Msum was best explained by a model ($F_{6,215} = 33.3$, $p < 0.0001$, $r^2_{adj} = 0.47$) including a third order function of both temperature and absolute humidity (figure 2.3, table 2.2). The relationship between residual Msum and minimal temperature (model including only temperature effect: $r^2 = 0.44$) followed a Z-shape characterized by a minimal average Msum of 1.23 W reached at 24.0°C and a maximal average Msum of 1.55 W reached at -10°C. Therefore the amplitude of Msum flexibility was of 0.32 W and the slope, calculated in the linear part of the curve (between 2°C and 14°C) was of -0.013 $W.°C^{-1}$ (figure 2.3a). The relationship between residual Msum and residuals of minimal absolute humidity followed a U-shape, with the lowest Msum reached at an absolute humidity of 2.8 $g.m^{-3}$ (figure 2.3b). However, although the effect was significant, the amount of variation explained by humidity was small since the addition of residual absolute humidity to the model

only increased r^2 by 0.04 (model including temperature and humidity effects: $r^2 = 0.48$). Consequently, individual birds expressed similar mass and sex independent Msums, which increased curvilinearly with a decline in ambient temperature and, for a given temperature, residual Msum tended to be higher when the amount of water vapour contained in the air was both low and high within the measured range.

Table 2.2 Best model explaining mass and sex independent Msum variations. Residual minimal absolute humidity was extracted from a polynomial regression and controls for the effect of ambient temperature.

Parameters	Estimate	Standard error	F	p
Intercept	-0.01940	0.01411		
Tmin	-0.01665	0.00177	151.6	<0.0001
Tmin^2	-0.00041	0.00007	19.2	<0.0001
Tmin^3	0.00002	0.00001	10.6	<0.01
resAHmin	-0.01382	0.01559	7.8	<0.01
resAHmin^2	0.04464	0.01390	6.3	<0.05
resAHmin^3	0.01104	0.00513	4.6	<0.05

Tmin: minimal ambient temperature (°C)
resAHmin: residual minimal absolute humidity ($g.m^{-3}$)

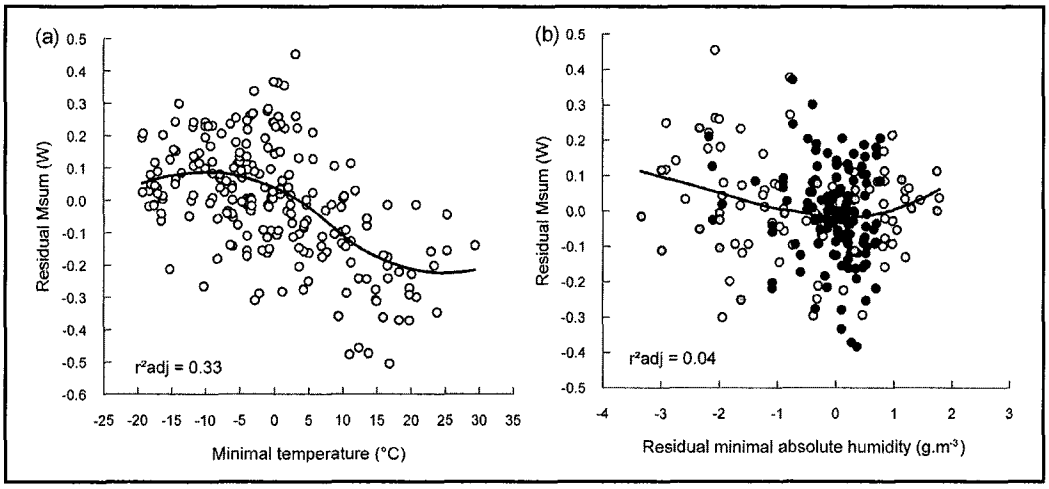

Figure 2.3 Mass and sex independent Msum variation over the natural range of weather conditions. Data are residual Msum presented against (a) minimal ambient temperature (min Ta) and (b) residual minimal absolute humidity. Black dots in (b) represent data collected at min Ta < 0°C, white dots at 0°C<min Ta<16°C and grey dots at min Ta>16°C (see text for details). For both panels, black lines are curves fitted by loess (smoothness = 0.85, degree = 2). Residual Msum were extracted from ANCOVA controlling for body mass, sex as well as residual minimal absolute humidity in (a) or minimal temperature in (b). Residual minimal absolute humidity controls for the effect of ambient temperature and was extracted from a polynomial regression. Coefficients of determination are presented for independent relationships, see text for complete model.

2.6 Discussion

Our goal was to investigate reaction norms of BMR and Msum through the natural range of weather variations experienced by a small endotherm. We expected non-linear responses, as metabolic rates would likely be limited by physiological constraints at the extremes of the range (McKechnie, 2008). We found that, in Black-capped chickadees, mass and sex independent BMR was explained by variation among individuals and was linearly related to temperature. In contrast, mass and sex independent Msum did not vary among individuals but was related to ambient temperature following a Z-shape relationship, with a linear increase between 24°C and -10°C, and to absolute humidity following a U-shape curve.

2.6.1 BMR

Mass and sex independent BMR was negatively correlated with minimal ambient temperature, suggesting that physiological maintenance costs in chickadees increases as ambient temperature decreases, a finding consistent with previous observations in other model species (Broggi *et al.*, 2007; Swanson & Olmstead, 1999). The relationship was linear over the experienced range of minimal ambient temperatures. This implies that, although the natural variation in temperature was relatively wide (-27°C to +32°C, see figure 2.4), BMR did not reach its minimal or maximal limits within this range. This therefore suggests that the range of flexibility in chickadee's BMR is wider than what has been recorded here and previous studies support this interpretation. Indeed, we reported earlier a seasonal increase in chickadee's BMR of only 6% in winter relative to summer for the same year at this location (Petit *et al.*, 2013) while Sharbaugh (2001) and Cooper and Swanson (1994) found seasonal differences of 12% and 14% for Alaska and South Dakota populations respectively. Interestingly, at those locations, the natural range of ambient temperature is not much wider (-30°C to +20°C, Sharbaugh 2001) and -10°C to +33°C, Cooper & Swanson 1994) than what we reported in this study. Combined with other studies that observed lower BMR in winter relative to summer (Saarela *et al.*, 1995; Smit & McKechnie, 2010), our results therefore suggest that the constraints driving the BMR reaction norm in Black-capped chickadees is unlikely to result directly from thermoregulatory requirements.

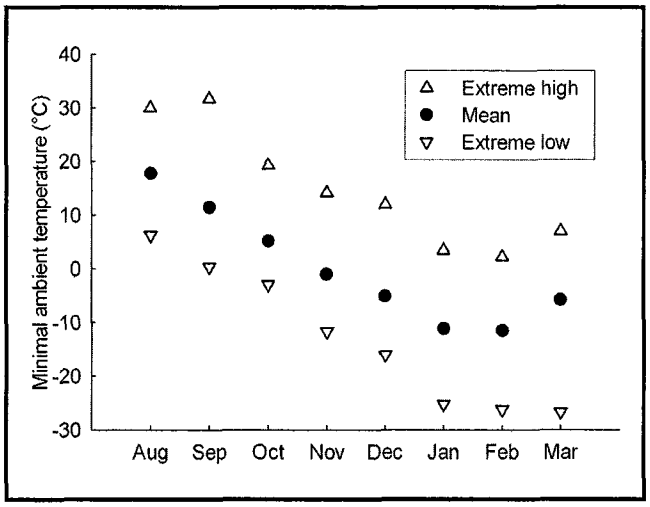

Figure 2.4 Monthly variations in minimal temperature. Data are extreme low, mean and extreme high values per month for minimal ambient temperature.

Variations in mass and sex independent BMR were also explained by differences among individuals in elevations. Individuals expressed the same rate of change in residual BMR (no effect of individual slopes) and residual BMR was repeatable ($R = 0.20$) over the measured range of temperature. Therefore, it appears that some individuals consistently maintained a higher BMR relative to others, which suggests that there might be differences among individuals in terms of investment in physiological maintenance (scenario 2), at least in that specific year. Whether this may result in fitness differences among individuals, in which case BMR would reflect individual quality (Wilson & Nussey, 2010), remains to be investigated.

2.6.2 Msum

Mass and sex independent Msum was related to both minimal temperature and absolute humidity. The correlation between minimal temperature and residual Msum plateaued below -10°C and above 24°C. Between these extremes, chickadees increased their maximal heat production capacity with decreasing ambient temperature. Our temperature data (figure 2.4) shows that ambient temperature in August can be as low as 6.3°C while average minimal ambient temperature is already below the 24°C threshold at this time and location. By October,

the warmest minimal temperature never reaches this value. This implies that by the end of August, time at which temperatures are still relatively warm, chickadees are already beginning to improve their thermogenic capacity in preparation for the incoming cold conditions. This interpretation goes in hand with our earlier observations (Petit *et al.*, 2013) where we demonstrated that Msum in chickadees from this population had already reached 22% of its inter-seasonal cold acclimatized level by October. This finding also suggests, assuming that the norm of reaction for mass and sex independent Msum is constant across populations, that chickadees from the northernmost populations, such as those found in Alaska, may never be able to reduce their thermogenic capacity to its lowest level since maximal summer ambient temperatures typically remains below 20°C at this latitude (Sharbaugh, 2001). These birds may therefore be forced to maintain a certain level of cold acclimatized phenotype even at the warmest time of the year (Olson & Grubb, 2007).

Chickadees reached their maximal population average residual Msum at a mean minimal temperature of -10°C. This value is well above the lowest temperatures recorded during the coldest months of winter at our site (often < -20°C, see figure 2.4) but matches with the average minimal temperature measured at that time (Jan: -11.2°C; Feb: -11.5°C) (figure 2.4). Therefore, by the time minimal ambient temperature averaged around -10°C, birds had reached the level of thermogenic capacity that seems to be required for surviving the winter. This suggests that chickadees' thermogenic capacity is adjusted to average environmental conditions rather than to acute temperature extremes.

The highest level of mass and sex independent Msum measured here could represent a physiological and/or a morphological limit (McKechnie, 2008). For example, pectoral muscles could be at their maximal size preventing further increases in shivering capacity at temperatures below -10°C (Petit & Vézina, 2014a; Swanson *et al.*, 2013). Hence, to face temperature colder than -10°C, birds would have to use strategies such as microhabitat selection (Wolf & Walsberg, 1996) or hypothermia (Lewden *et al.*, 2014; Sharbaugh, 2001) to compensate for the lack of endogenous heat production. However, one has to keep in mind that Msum is only an indicator of cold endurance (Swanson, 2001) and the level of thermogenic capacity that birds reach when average minimal temperature is around -10°C should be sufficient to support short-term events of temperatures below -10°C. The maximal level of Msum reported here (1.55 W) is similar to

what has been observed in Black-capped chickadees from Ohio and Wisconsin (both 1.5 W, Olson *et al.*, 2010) but is lower than that reported for South Dakota (2.1 W, Olson *et al.*, 2010). Determining to what level Msum can increase above the plateau documented here and the influence of ambient conditions in setting maximal Msum will require further investigation.

Mass and sex independent Msum was also related to residual absolute humidity following a U-shape pattern. For a given ambient temperature, residual Msum was higher when the air contained both relatively low and high amounts of water vapour. Dry air favours evaporation and the loss of body heat (Webster & King, 1987). It is therefore not surprising to see that birds tended to maintain a higher thermogenic capacity when conditions were dry for a given temperature. In contrast, finding elevated Msums at high levels of humidity came as a surprise as one would expect the effect of humidity on heat loss to be negligible in the cold due to condensation. However, it is worth noting that 43% of our sample has been collected during days where minimal ambient temperature was above 0°C (figure 2.3b) and 35% of our measures were obtained when temperatures were above the freezing point but still below the lower critical temperature for chickadees (16°C in winter and 19.9°C in summer, Cooper & Swanson, 1994). It is therefore conceivable that at temperatures above 0°C, water vapour increased heat transfer through air and thus contributed to increase body heat loss and, consequently, individual's maximal thermogenic capacity. We nevertheless must emphasize that the number of observations at high levels of humidity, where residual Msum was found to increase, were relatively few and contained measurements on days where temperatures were above the lower critical temperature (figure 2.3b). Although the cooling effect of water vapour at these temperatures was apparently sufficient to trigger an upregulation of Msum, this interpretation must nonetheless be considered with caution and requires further investigations.

Residual Msum varied with temperature and humidity but did not differ among individual birds. Therefore, for a given mass and sex, individual chickadees expressed similar variations in Msum throughout the year (scenario 3). As maximal thermogenic capacity is thought to reflect long-term cold endurance (Swanson, 2001) and survival (Jackson *et al.*, 2001), this suggests that Msum could be under stabilizing selection pressure, which would work to eliminate extreme phenotypes. Theoretically, individuals with low levels of Msum may not be able to survive episodes of prolonged cold spells while constantly maintaining a higher capacity than required

might reduce available resources for other fitness-related physiological functions such as immunity (Eraud *et al.*, 2005) or defence against oxidative damage (Costantini & Dell'Omo, 2006). Such stabilizing selection would lead to a low degree of individual variation in Msum as we observed here, although, this may only be true for specific sets of winter conditions as recent evidences suggest that repeatability of Msum may be year-specific (Cortés *et al.*, 2015). Alternatively, while following the general Z-shape pattern, residual Msum could also be highly variable within individuals, which would prevent us from detecting any Msum consistency and therefore from highlighting between-individual differences.

To the best of our knowledge, despite a relatively small recapture sample size (see Martin *et al.*, 2011 for suggested requirements with random regressions), this study is the first to investigate reaction norms of physiological parameters (*i.e.* metabolic performance) over the natural range of weather conditions in small free-living endotherms, both at the individual and population levels. We demonstrated that physiological maintenance costs were linearly related with ambient temperature but not limited within the measured range and were individual-dependent in our population. In contrast, heat production capacity, and thus cold tolerance, was not dependent on individuals but was related to both ambient temperature and absolute humidity following cubic relationships potentially highlighting limits to physiological adjustments. This supports the assertion that basal and maximal cold-induced metabolic rates are functionally uncoupled and that phenotypic flexibility of these traits may be responding to different and possibly independent constraints (Petit *et al.*, 2013).

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CHAPITRE III

Les manipulations phénotypiques confirment l'implication des muscles pectoraux et de l'hématocrite dans la capacité thermogénique maximale chez les oiseaux

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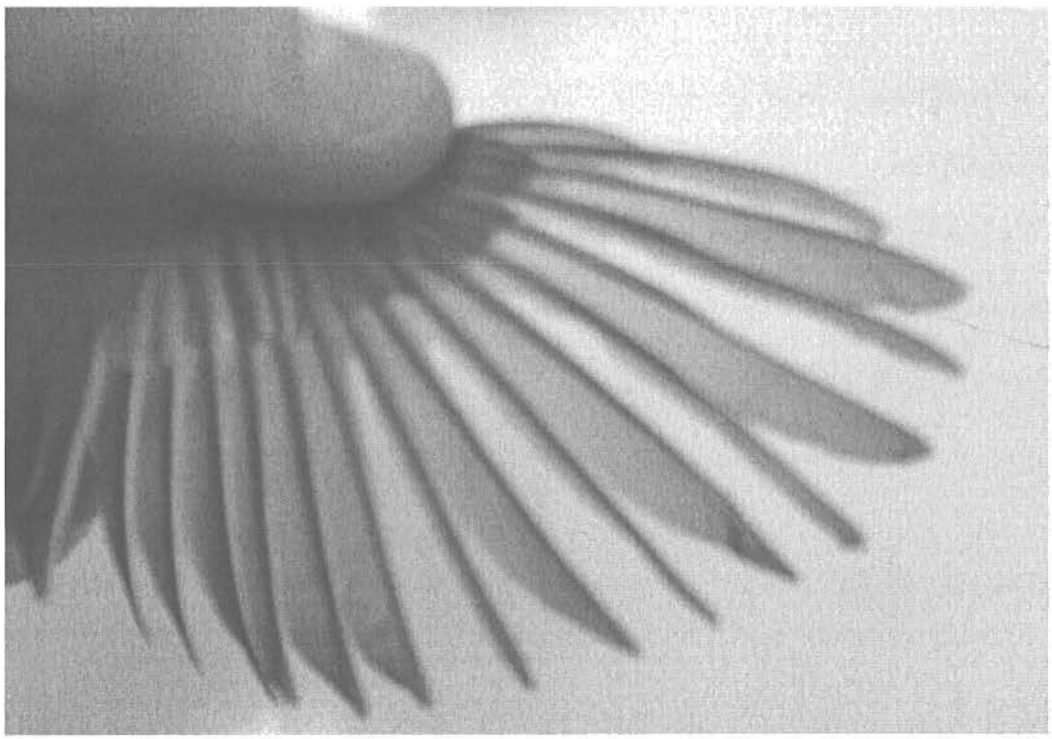

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Phenotype manipulations confirm the role of pectoral muscles and hæmatocrit in avian maximal thermogenic capacity

Magali Petit, François Vézina

Département de biologie, chimie et géographie, Université du Québec à Rimouski, Rimouski (QC), Canada. Groupe de recherche sur les environnements nordiques BORÉAS, Rimouski, (QC), Canada. Centre d'Études Nordiques, Québec (QC), Canada. Centre de la Science de la Biodiversité du Québec, Montréal (QC), Canada.

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Contributions - Conception de l'expérience: MP, FV ; Réalisation de l'expérience: MP; Analyse des données: MP ; Financement: FV ; Rédaction : MP, FV.

3.1 Résumé

Pour les espèces d'oiseaux de petite taille qui résident à de hautes latitudes, l'acclimatation hivernale est caractérisée par une augmentation de la taille des muscles pectoraux et de l'hématocrite, ce qui permettrait la hausse saisonnière du métabolisme maximal induit par le froid (Msum, une mesure de la capacité maximale à produire de la chaleur par frissonnement). Cependant, les évidences supportant une relation entre la taille des muscles pectoraux et le Msum ne sont que corrélatives et le lien entre l'hématocrite et le Msum reste à démontrer. Nous avons testé expérimentalement la relation entre la taille des muscles pectoraux et le Msum en manipulant la taille des muscles par un protocole de réduction de surface alaire appliqué au sein d'une population naturelle de mésanges à tête noire (*Poecile atricapillus*). Ce protocole nous a aussi permis de tester le lien entre l'hématocrite et la capacité thermogénique. Après une première série de mesures sur tous les oiseaux, nous avons coupé la moitié des plumes de vol chez les individus du groupe expérimental (n = 14). Nous avons ensuite comparé le niveau d'adiposité, la taille des muscles pectoraux, le Msum et l'hématocrite mesurés à la recapture avec les valeurs obtenues lors de la première capture et avec les valeurs obtenues chez des individus du groupe contrôle (n = 17) capturés et recapturés à des périodes similaires. Les résultats ont montré 1) que les oiseaux expérimentaux ont développé de plus gros muscles pectoraux que les oiseaux contrôles et 2) que le Msum corrigé pour la masse était jusqu'à 16% plus élevé chez les oiseaux arborant de gros muscles. Le Msum était aussi positivement corrélé à l'hématocrite qui, lui, n'était pas affecté par la manipulation expérimentale. Cette étude démontre que chez les mésanges à tête noire avoir de gros muscles pectoraux pour une masse donnée est associé à un Msum élevé et que la capacité de transport de l'oxygène supporte probablement la thermogénèse chez cette espèce.

Mots-clefs: capacité thermogénique maximale ; frissonnement ; performance métabolique ; feather clipping ; hématocrite.

3.2 Abstract

In small resident bird species living at northern latitudes, winter cold acclimatization is associated with an increase in pectoral muscle size and hæmatocrit level and this is thought to drive the seasonal increase in summit metabolic rate (Msum, a measure of maximal shivering thermogenic capacity). However, evidences suggesting that pectoral muscle size influences Msum are correlational and the link between hæmatrocrit level and Msum remains to be demonstrated. We experimentally tested the relationship between pectoral muscle size and Msum by manipulating muscle size using a feather clipping protocol in free-living wintering Black-capped chickadees (*Poecile atricapillus*). This also allowed us to investigate the link between hæmatocrit and thermogenic capacity. After a first series of measures on all birds, we cut half of the flight feathers of experimental individuals (n = 14) and compared their fat and pectoral muscle scores, Msum and hæmatocrit level at recapture with their previous measures and with those of control birds (n = 17) that were captured and recaptured at comparable times. Results showed 1) that experimental birds developed larger pectoral muscles than control individuals and 2) that mass-independent Msum was up to 16% higher in birds expressing large pectoral muscles. Msum was also positively correlated with hæmatocrit, which was not affected by the experimental manipulation. These findings demonstrate that, for a given body mass, large pectoral muscles are associated with a higher Msum in Black-capped chickadees and that oxygen carrying capacity likely supports thermogenesis in this species.

Keywords: maximal thermogenic capacity; shivering; metabolic performance; feather clipping; hæmatocrit.

3.3 Introduction

Birds overwintering at northern latitudes face low ambient temperatures and restricted food availability (McNamara & Buchanan, 2005; McNamara *et al.*, 1990; Swanson, 2010). To survive in this environment, a suite of physiological traits must adjust to winter conditions (Cooper & Swanson, 1994; Swanson, 1991a; Swanson & Thomas, 2007). Specifically, winter acclimatization is associated with an increase in body mass (Carey *et al.*, 1978; Vézina *et al.*, 2006; Williams & Tieleman, 2000), fat reserves (Cooper, 2002; Sharbaugh, 2001; Swanson, 1991a), muscle mass (Cooper, 2002; Liknes & Swanson, 2011b; Swanson, 1991b), summit metabolic rate (Msum) (Cooper & Swanson, 1994; Swanson, 1991a; Vézina *et al.*, 2006) and hæmatocrit (deGraw *et al.*, 1979; O'Connor, 1996; Swanson, 1990b).

Msum is a measure of maximal thermogenic capacity and individuals with higher Msums have been shown to express better cold tolerance (Swanson, 1990a) and endurance (Swanson, 2001), meaning that they can face colder temperature over longer periods than birds with lower Msum. Because pectoral muscles are the largest muscles in birds (Marsh & Dawson, 1989; O'Connor, 1995; Swanson, 1991b), it is widely assumed that the increase in Msum typically seen in cold acclimatized birds (Cooper, 2002; O'Connor, 1995; Petit *et al.*, 2013) results from the associated increase in pectoral muscle size (Cooper, 2002; Saarela & Hohtola, 2003; Vézina *et al.*, 2011; Vézina *et al.*, 2007) and recent findings by Swanson *et al* (2013) in American goldfinches (*Spinus trisis*) support this interpretation. However, the link between Msum and the size of pectoral muscles remains correlative and the relationship has yet to be tested experimentally. For instance, maximal heat production could also benefit from non-shivering heat sources such as elevated tissue metabolic intensity (Vézina *et al.*, 2011; Zheng *et al.*, 2013a; Zheng *et al.*, 2013b) and cases of cold acclimation without changes in size of pectoral muscles have been reported (Vézina *et al.*, 2006; Williams & Tieleman, 2000).

Hæmatocrit (the proportion of red blood cells in total blood) is a measure of maximal oxygen carrying capacity, which is thought to support overall metabolic activity (Burness *et al.*, 1998; Calbet *et al.*, 2006; Carpenter, 1975; Hammond *et al.*, 2000), including thermogenesis (Carey & Morton, 1976; Swanson, 1990b). For instance, Swanson (1990b) found 11% higher hæmatocrit and 9% higher oxygen carrying capacity in dark-eyed juncos (*Junco hyemalis*) during winter relative to summer and showed in a parallel study that juncos also expressed elevated Msum in

winter (Swanson, 1990a). Winter increases in hæmatocrit are therefore interpreted as a physiological upregulation in response to elevated oxygen demands for thermogenesis (Carey & Morton, 1976; Swanson, 1990b), which likely maximizes heat production capacity and cold endurance. However, increasing the number of cells in circulation also increases blood viscosity, which suggests that the relationship between hæmatocrit and thermogenic capacity should not be linear but rather dome shaped with an optimal hæmatocrit found at intermediate levels (Schuler *et al.*, 2010), which may vary among seasons depending on heart size adjustments (*e.g.* Liknes and Swanson 2011). The functional link between Msum and hæmatocrit, however, remains to be demonstrated.

In this study, we manipulated the pectoral muscle size of free-living Black-capped chickadees (*Poecile atricapillus* [*Linnaeus* 1766]) by cutting the primary and rectrix feathers (Ardia & Clotfelter, 2007; Harding *et al.*, 2009b; Sanz *et al.*, 2000) of experimental individuals (figure 3.1). This technique reduces wing and tail surface area, which has been shown to force birds to develop larger pectoral muscles to compensate for the loss in lift (Lind & Jakobsson, 2001). Based on the known correlation between pectoral muscle size and Msum (Swanson *et al.*, 2013; Vézina *et al.*, 2007), we predicted that compared to "control" birds, "clipped" chickadees would develop larger pectoral muscles leading to an increase in Msum. Assuming that upregulating Msum would also require an increase in oxygen delivery (Carey & Morton, 1976; Swanson, 1990b), we expected a positive relationship between hæmatocrit and Msum and higher hæmatocrit in clipped birds relative to controls.

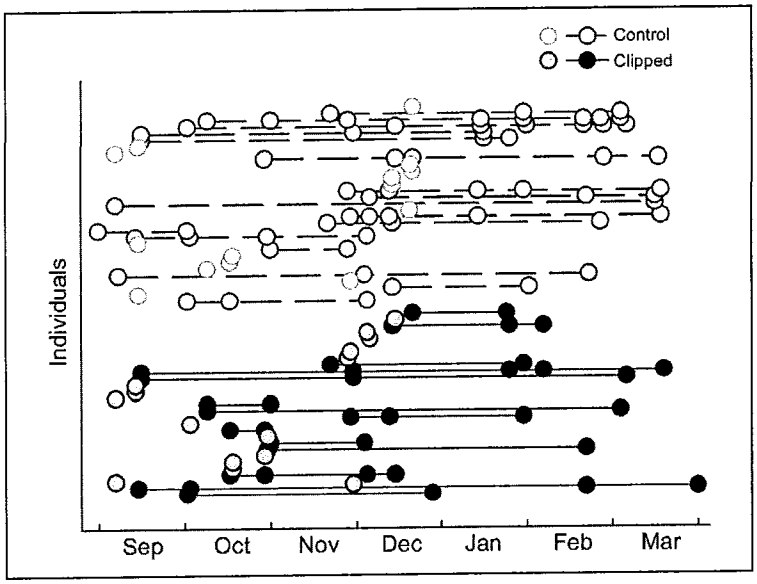

Figure 3.1 Captures and recaptures of Black-capped chickadees. Each individual capture is represented by a circle. Grey circles represent individuals that were not recaptured. Circles joined by a line represent captures and recaptures for given individuals.

3.4 Materials and methods

3.4.1 Capture and handling

Black-capped chickadees are small (9-14 g) non-migratory passerines that form territorial social groups during winter (Smith, 1993), which allows for tracking individuals over time relatively easily. This study was carried out in the Forêt d'Enseignement et de Recherche Macpès, Québec, Canada (48°18' N, 68°31' W), where we caught 60 free-living individuals between September 2010 and March 2011 (average minimal temperature recorded hourly over the study period: -2.5 ± 0.1°C; see table 3.1 for details). Sixteen feeding stations were set-up, each including three metal feeders filled with black sunflower seeds and two wooden poles that were topped with plastic trays (Lewden *et al.*, 2012; Mandin & Vézina, 2012). On capture days, we removed feeders and put a potter-trap (15 cm x 15 cm x 15 cm) baited with black sunflower seeds on each tray. All birds were caught between 08:00 and 13:00 and, following

capture, birds were banded with a USGS numbered metal band and a unique combination of three plastic color bands that allowed individual identification from a distance.

Table 3.1 Mean minimal ambient temperature (± s.e.m.) recorded hourly over the study period.

Month	Mean minimal Ta ± s.e.m. (°C)
September	11.44 ± 0.19
October	05.15 ± 0.14
November	-01.02 ± 0.15
December	-05.07 ± 0.19
January	-11.20 ± 0.25
February	-11.52 ± 0.23
March	-05.76 ± 0.23

For each captured bird, we collected a blood sample within less than 10 minutes by puncturing the brachial vein with a 26-gauge needle. Samples (total blood volume < 120 µl) were kept cold (< 4°C) in heparanized microcapillary tubes until centrifugation (10 min at 16 000 g) and hæmatocrit measurements. After blood sampling, birds were weighed and were measured following standardized protocols (length of beak, head plus beak, tarsus, tail and wing measured) (Lewden *et al.*, 2012; Mandin & Vézina, 2012; Petit *et al.*, 2013). The furcular fat stores were evaluated using a visual score from 0 (no visible fat) to 5 (fat overlapping pectoral muscles) according to Gosler (1996). Pectoral muscle size was determined on a scale of 0 (keel prominent and muscles depressed) to 3 (keel difficult to see and muscles rounded) (Busse, 2000). Although muscle score is a relatively crude index, it nevertheless reflects differences in pectoral muscle mass. Indeed, we dissected pectoral muscles of 79 chickadees for another study and observed a positive relationship between muscles score and size-corrected lean dry mass of pectoral muscles ($F_{2,75} = 29.2$, $p < 0.0001$; $r^2adj = 0.45$). Birds with muscles scored as 3 had pectoral muscle on average 21% heavier than birds scored as 2 and these latter individuals had pectoral muscles 18% heavier than those scored as 1 (MP and FV, unpublished data).

The Black-capped chickadee is a monomorphic species. We were therefore unable to determine the sex of individuals for this study. For each day of capture, a maximum of four birds were brought to the station for Msum measurements. All bird manipulations were

approved by the animal care committee of the Université du Québec à Rimouski and have been conducted under scientific and banding permits from Environment Canada - Canadian Wildlife Service.

3.4.2 Respirometry

At the station, birds were maintained in individual cages (39 cm x 43 cm x 31 cm) with food (sunflower seed) and water provided *ad libitum* prior to Msum measurements. Within 24 hours from capture, we measured their Msum between 13:00 and 18:00 and released the individuals at their original catching site after having performed the experimental manipulation (see below).

We measured the Msum of two birds at the same time using two FoxBox oxygen analyzers (Sable Systems, Las Vegas, NV, USA) and following the protocol described by Lewden *et al.* (2012) and Petit *et al.* (2013). Before the Msum measurement, we weighed (± 0.1 g) each individual and measured their body temperature with a copper-constantan thermocouple inserted into the cloacae approximately 10 mm deep (NIST-traceable thermocouple reader Omega model HH-25KC). Birds were then placed in a stainless steel metabolic chamber (effective volume = 1120 ml) and received a mixture of 21% oxygen and 79% helium (helox) using an average flow rate of 1109 $ml.min^{-1}$ controlled by mass flow valves (Sierra Instruments, Side-Trak® Model 840, Monterey, CA, USA). We used a sliding cold exposure protocol (Swanson *et al.*, 1996) during the Msum trials where ambient temperature was ramped down by 3°C every 20 minutes, starting at 0°C. Trials ended when birds became hypothermic, which was easily detectable from the O_2 readings. Body temperature and body mass were measured again at the end of each trial. Only data from birds showing a body temperature after trials lower or equal to 38°C (Cooper & Gessaman, 2005) were used and average body mass was used in the statistical analyses. Msum values were calculated using the highest averaged 10 minutes of oxygen consumption calculated with the instantaneous measurement technique (Bartholomew *et al.*, 1981). Since birds use lipids as metabolic fuel during shivering (Swanson, 2010) we estimated energy consumption in Watts using a constant equivalent of 19.8 $kJ.L^{-1}O_2$ (Gessaman & Nagy, 1988).

Mass flow valves were calibrated for helox with a bubble-O-meter (Dublin, OH, USA) prior to the experiment and oxygen analyzers were adjusted before each Msum trial with CO_2-free dry air. Calculations of metabolic rates were done using EXPEDATA software, v1.2.6 (Sable Systems, Las Vegas, NV, USA) according to equation 10.1 from Lighton (2008).

3.4.3 Experimental treatments

From September to December 2010, we used a feather clipping protocol to manipulate pectoral muscle size. Following respirometry measurements, we randomly assigned birds to one of two experimental treatments: "clipped" or "control". Clipped birds had half of their flight feathers cut along the main shaft (*i.e.* removing barbs and barbules) on both wings (primaries P1-P3-P5-P7-P9) and both sides of the tail (rectrices R2-R4). This approach prevents premature regrowth of cut feathers (P. Perret, pers.com) and insures long lasting effects of the treatment until the next molt (Harding *et al.*, 2009a; Sanz *et al.*, 2000). Control individuals were handled similarly to clipped birds but their feathers were not clipped. Following Msum measurement, half of the birds were assigned to the clipped group while the other half was assigned to the control group. Over the 60 birds (clipped: n = 29; control: n = 31) initially caught and measured, we obtained a final sample size of 14 clipped and 17 control individuals (recapture rate = 55% for control birds and 48% for clipped birds) (figure 3.1).

3.4.4 Statistical analysis

3.4.4.1 Testing for the effect of duration between captures and recaptures

This protocol was carried-out in natural conditions and this resulted in variable numbers of recapture per individual (figure 3.1). However, there was no difference in the average number of recapture between treatments (clipped: 1.9 ± 0.4, control: 2.7 ± 0.4, p = 0.1) and the average duration between first capture (*i.e.* the day of manipulation) and all recaptures for a given bird also did not differ between treatments (79.6 ± 10.4 days for clipped birds and 84.1 ± 7.5 days for control birds p = 0.3). To determine whether the duration between captures could create a

significant effect on our dependent variables, we used data from individuals for which we had at least one recapture and tested for an effect of the duration between the day of manipulation and the day of each capture. This was done using linear mixed effect (LME) models for linear variables (body mass, hæmatocrit and Msum) and ordinal random effect regressions (Christensen, 2013) for ordinal parameters (fat and muscle scores). Models tested for the effect of the duration while controlling for date of capture, group (clipped or control), the interaction "group by duration" and bird ID as a random parameter. However, Msum is affected by body mass and body mass is affected by structural body size and daytime due to daily fattening (Mandin & Vézina, 2012). We therefore performed a principal component analysis on morphological data (length of head plus beak, wing and tarsus) to use the first principal component as a measure of structural body size (thereafter called "size") (Rising & Somers, 1989) and we also calculated relative time (time since sunrise / day length, hereafter "time of capture") for each capture. We could then analyse the effect of the duration between the captures on size-independent body mass and mass-independent metabolism by adding size and time of capture or mass as covariates in LME models. In all of these analyses, we used a likelihood ratio test (LRT) to compare the complete models to models without the effect of the duration between captures. Duration had no significant effect on any of our dependent variables (size-independent body mass: $p = 0.5$, hæmatocrit: $p = 0.6$, mass-independent Msum: $p = 0.2$, fat score $p = 0.2$ and muscle score $p = 0.9$). We therefore did not include the duration between captures in further analyses and rather encoded our data according to two periods. The period called "before" designated data collected at the first capture (before applying the treatment, between September and December with 62% of the first captures done between September and October) while the period called "after" designated all measurements collected each time a bird was recaptured (individuals were recaptured from 1 to 7 times with an average of 2.3 ± 0.3 times between October and March, 59% of all the recaptures happened between January and March). Because date of capture was redundant with period, we also removed the variable "date of capture" from further analyses.

3.4.4.2 Testing for treatment effects on dependent variables

We began our analyses by testing whether control and clipped individuals were different at first capture (*i.e.* before manipulation, n = 60). We used general linear models or ordinal regressions to test for a group effect on body mass, hæmatocrit, Msum, fat score and muscle score while considering the potential effect of date and time of first capture as well as body size or body mass when appropriate (see above). We then investigated whether individuals that were later recaptured were differing in values of these variables relative to birds that were not recaptured. This was done by including the variable "recapture" (yes or no) in the models.

To test for the treatment effect, only data from birds that were recaptured at least once were used. Fat and muscle scores were analysed by ordinal random effect regressions to track changes in fat stores and muscle size over the winter. Ordinal regressions considered the effects of periods (before or after treatment), groups, the interaction term period*group and bird ID as a random parameter to account for repeated measures. The effect of each variable was determined using the LRT method (Christensen, 2013). Fat and muscle scores were measured by 4 observers (66% of the observations done by M.P) but when considered in models, the effect of observers on fat score and muscle score were not significant ($p = 0.1$ and 0.3 respectively). We therefore did not consider this effect in our analyses.

To analyse winter variations of body mass, Msum and hæmatocrit according to periods, groups and the interaction term period*group, we ran LME models including bird ID as a random variable. We controlled for the effect of size and time of capture on body mass and mass on Msum by including these variables as covariate in respective models. Muscle score and hæmatocrit were also added to the Msum model to study the effect of pectoral muscle size on mass-independent Msum and the potential relationship between hæmatocrit and thermogenic capacity. Since we expected a parabolic relationship between Msum and hæmatocrit, we considered the quadratic relationship between these variables by including a second order polynomial function for the hæmatocrit effect in the LME.

Ordinal regressions were performed with R (R Core Team, 2013) using the ordinal package (Christensen, 2013). *Post-hoc* analyses were performed using Tukey tests on least square means computed from LMEs. We removed non-significant variables from models and final results are

presented here as least square means ± s.e.m. for linear parameters and as medians for ordinal variables. Residuals were tested for normality using the one sample Kolmogorov-Smirnov test.

3.5 Results

3.5.1 Treatment effect

On the day of first capture none of the dependent variables we investigated differed among future experimental treatments (group effect: $p = 0.1$-0.8). Similarly, individuals that were recaptured later did not differ in any of these variables at first measure from individuals that were never seen again ($p = 0.5$-0.9).

Average body mass was not affected by size ($p = 0.2$) and did not differ between groups ($p = 0.1$) but did vary with time of capture ($F_{1,78} = 5.6$, $p < 0.05$) and period ($F_{1,69} = 5.8$, $p < 0.05$). The interaction term period*group was also close to significance ($F_{1,68} = 3.4$, $p = 0.07$) indicating a trend where control individuals gained body mass between periods while clipped birds did not (figure 3.2). Independent analyses per group confirmed this trend, showing that control birds were on average 2.9% heavier after the treatment than before ($F_{1,45} = 15.0$, $p < 0.001$) while clipped birds maintained a constant body mass throughout the winter ($p = 0.9$) (figure 3.2).

Fat scores did not vary between periods ($p = 0.5$) or groups ($p = 0.09$) and were not affected by the period*group interaction ($p = 0.7$).

Analysis of pectoral muscle score showed that both groups ($X^2 = 6.1$, $df = 1$; $p < 0.05$) and periods ($X^2 = 5.7$, $df = 1$; $p < 0.05$) were significant but that the group effect also tended to depend on the period (period*group: $p = 0.07$). Clipped birds had larger muscles on average (median = 3) than controls (median = 2). However, when considering the interaction trend, at first capture both control and clipped individuals expressed a median muscle score of 2. After the treatment, clipped birds had larger pectoral muscles (median = 3) compared to control birds measured either before or after the treatment (median = 2) (figure 3.3). Therefore the significant group effect seemed driven mainly by an increase in pectoral muscle size in the clipped group between periods.

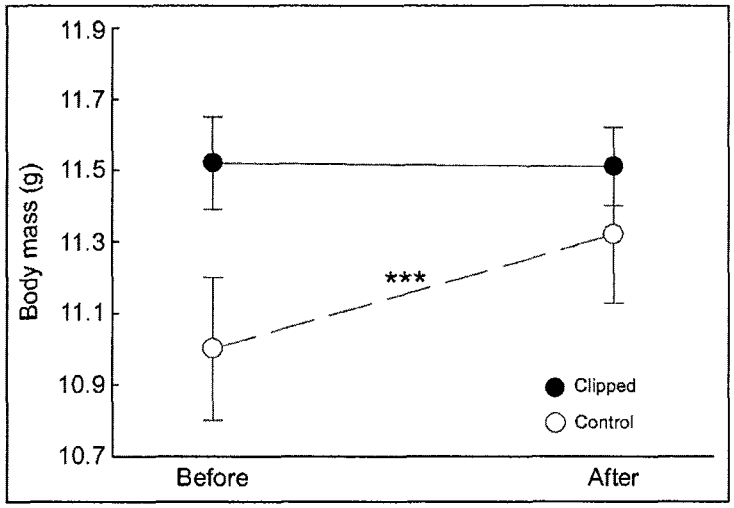

Figure 3.2 Variation in body mass over the experimental period. Data are least square means of body mass for control and clipped Black-capped chickadees before and after the treatment. Least square means were extracted from separated LME models per group controlling for period, body size and time of capture, with bird ID as random parameter. Asterisk indicates the significant increase in body mass within the control group between periods (*** $p < 0.001$).

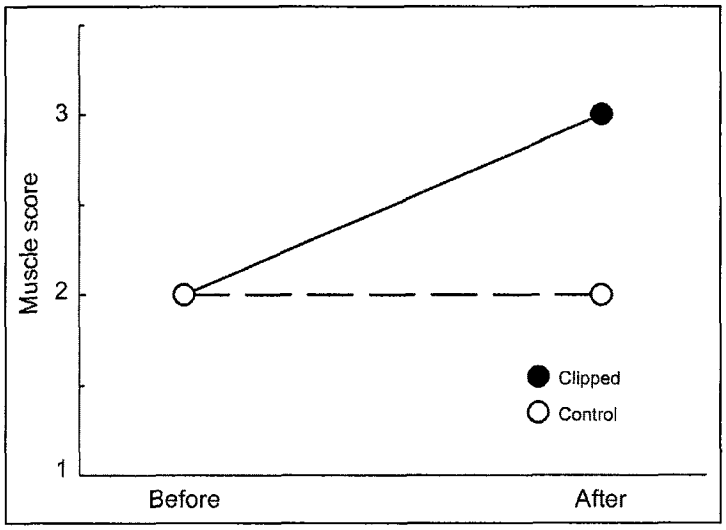

Figure 3.3 Variation in muscle score over the experimental period. Data are median muscle scores for control and clipped Black-capped chickadees measured before and after the treatment (as these are median values, there are no error bars).

Whole Msum was influenced by the period ($F_{1,51} = 38.5$, $p < 0.0001$) and group ($F_{1,24} = 5.4$, $p < 0.05$) with Msum being 16.7% higher after the treatment than before and with clipped birds having a Msum 7.8% higher than control birds (no significant interaction, $p = 1.0$). However, when body mass was included in the model ($F_{1,33} = 12.8$, $p < 0.01$) there was no difference between groups in Msum ($p = 0.2$). Mass-independent Msum was 14.7% higher after treatment than before (period: $F_{1,58} = 29.9$, $p < 0.0001$) (figure 3.4A).

Haematocrit was not affected by group ($p = 0.5$) or the interaction period*group ($p = 0.4$) but was dependent on the period ($F_{1,76} = 38.2$, $p < 0.0001$). Birds expressed an average hæmatocrit 8.5% higher after treatment than before (figure 3.4B). Haematocrit was independent of body mass ($p = 0.7$).

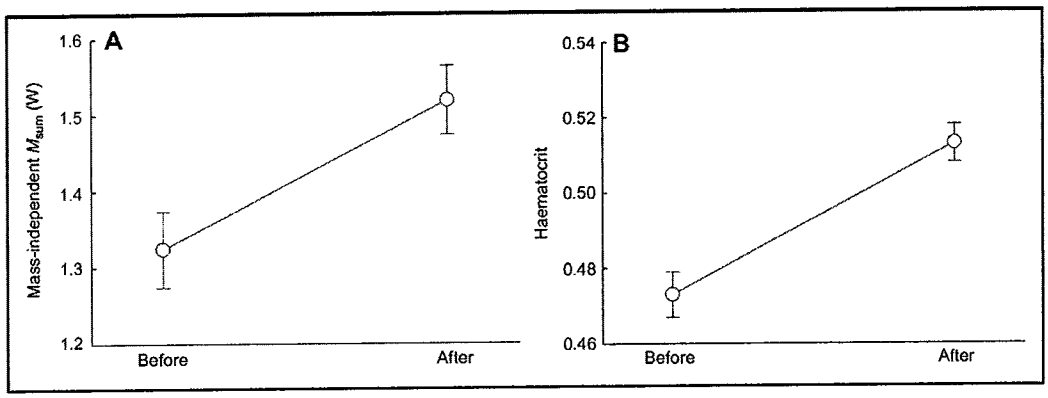

Figure 3.4 Changes in thermogenic capacity and haematocrit in Black-capped chickadees over the experimental period. Data are least square means of (A) summit metabolic rate (Msum) and (B) haematocrit before and after the treatment. Least square means were extracted from an LME model controlling for group, period and the interaction term group × period, with bird ID as random parameter. Body mass was also included in the model as a covariate for Msum.

3.5.2 Effect of pectoral muscle size and hæmatocrit on metabolism

When including hæmatocrit and muscle scores in LMEs, variations in mass-independent Msum were still affected by the period ($F_{1,69} = 4.3$, $p < 0.05$, 6.9% higher after manipulation) and remained independent from the group effect ($p = 0.7$). However, mass-independent Msum was positively related to the hæmatocrit level ($F_{2,69} = 10.1$, $p < 0.0001$; figure 3.5) and was affected by muscle score ($F_{2,69} = 4.1$, $p < 0.05$; figure 3.6). Therefore, for any experimental group, birds

with higher hæmatocrit and larger pectoral muscles had a higher Msum on average than individuals with lower values of both variables. Specifically, compared to individuals with muscle score of 1, Msum was 14.4% higher in birds with a muscle score of 2 (Tukey: $p < 0.05$) and 16.5% higher in birds with a muscle score of 3 (Tuckey $p < 0.05$) (figure 3.6). However, studying intra-individual changes in muscle score for each group revealed that over the study period, 50% of the clipped individuals (seven individuals out of 14) increased their pectoral muscle to a median score higher than 2 compared to only 29% (five individuals out of 17) for the control birds (figure 3.7). Thus, although the group effect was not significant for Msum in this particular analysis, the clipped group contained more individuals with the highest muscles score during the after period.

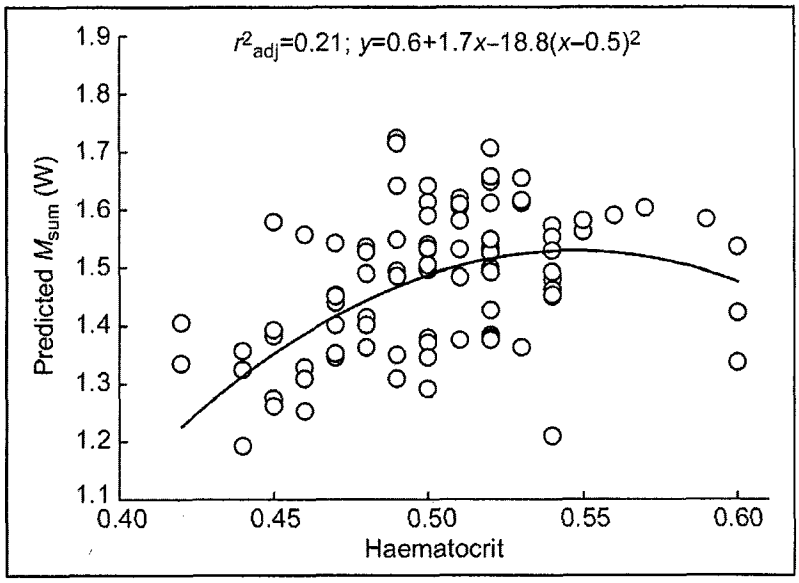

Figure 3.5 Relationship between Msum and haematocrit in Black-capped chickadees. Data are predicted Msum values plotted against haematocrit. Predicted values were computed from a LME model controlling for group, period, the interaction term group × period, muscle score and body mass as covariates, with bird ID as a random parameter.

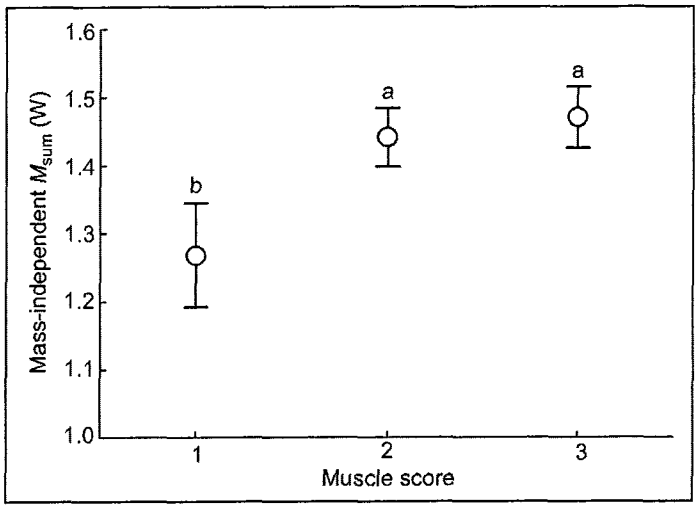

Figure 3.6 Relationship between Msum and pectoral muscle score in Black-capped chickadees. Msum data are least square means extracted from an LME model controlling for group, period, the interaction term group × period, body mass and haematocrit as a covariates, with bird ID as random parameter. Different letters indicate significant differences ($p < 0.05$).

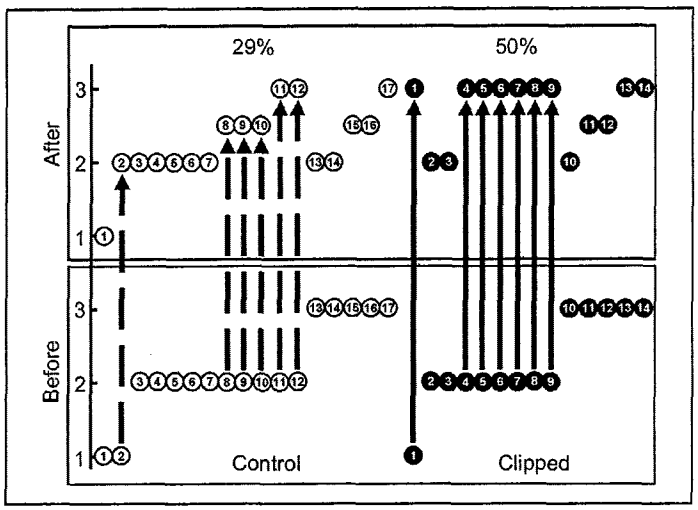

Figure 3.7 Individual changes in muscle scores between periods. The percentage of Black-capped chickadees increasing their muscle size to a score higher than 2 is presented above each group. Muscle scores presented for the after period are median values of all scores measured for a given bird over all its recaptures. Therefore, values for this period may include decimals. Arrows highlight birds that increased their muscle score between periods.

3.6 Discussion

The seasonal increase in thermogenic capacity commonly reported for wintering birds is often interpreted as the result of an enlargement of shivering muscles (Cooper, 2002; Saarela & Hohtola, 2003; Vézina *et al.*, 2011; Vézina *et al.*, 2007). This can be associated with an elevated hæmatocrit (Swanson, 1990b) interpreted as an upregulation of oxygen carrying capacity. However, evidences linking muscle size to Msum remain correlative (Cooper, 2002; Swanson *et al.*, 2013; Vézina *et al.*, 2006) and the link between hæmatocrit and Msum has not been demonstrated. In this study, we experimentally enlarged pectoral muscle size by clipping flight feathers and observed (1) that birds with larger pectoral muscles, including a high proportion of clipped individuals, also had high Msum and (2) that for a given body mass and muscle size, hæmatocrit was indeed positively related to Msum. Results of our phenotypic manipulation therefore support previous correlative evidences for the role of modulating pectoral muscle size and oxygen carrying capacity in Msum adjustments.

On average, birds from all treatments increased their body mass, the size of their pectoral muscles, their Msum and their hæmatocrit throughout the experiment. These seasonal changes are part of the natural acclimatization response found in wintering Black-capped chickadees. Indeed, both Sharbaugh (2001) and Liknes & Swanson (2011b) showed increases in body mass in wintering chickadees relative to summer counterparts. This species is also known to improve its metabolic performance in winter relative to summer (Cooper & Swanson, 1994; Lewden *et al.*, 2012; Petit *et al.*, 2013). For instance, using birds from the same population, Petit *et al.* (2013) reported that chickadees elevated their winter Msum by 25% between the beginning (October) and the peak of winter (February). The observed development of pectoral muscle size is also part of the natural change in phenotype associated with winter acclimatization and has been reported in several species (Cooper, 2002; Liknes & Swanson, 2011b; O'Connor, 1995; Swanson, 1991b), including Black-capped chickadees (Liknes & Swanson, 2011b).

In contrast with control birds that showed an increase in body mass during the experiment, clipped individuals maintained theirs constant. They also had larger pectoral muscles than control birds, especially during the after period. Therefore, our feather manipulation not only succeeded in forcing clipped individuals to develop larger pectoral muscles, but it also appears

that it prevented these birds from increasing their body mass, as did the controls. One could argue that clipped individuals were already at an optimal body mass at first capture, explaining why we could not detect an increase in mass in this group. However, there was no significant difference in body mass between groups at first capture, which mainly took place in the early autumn (September-October). Given that chickadees typically show a seasonal fattening cycle (Lehikoinen, 1987) peaking at the coldest time of winter (*i.e.* February at our study site, Petit *et al.*, 2013), this is therefore very unlikely. Two potential causes could explain the lack of increase in body mass in clipped individuals. First, these birds could have faced elevated daily energy expenditure due to higher wing loading and could therefore have been unable to maintain the positive daily energy budget required for the seasonal fat accumulation (Lehikoinen, 1987). As the rate of recaptures was lower in the clipped group, individuals unable to maintain a balanced energy budget could have died as a result of the experiment or moved away to more profitable or less risky habitats. Second, clipped individuals could have maintained constant body mass throughout winter, as a consequence of higher wing loading, in order to minimise flight costs and maintain manœuvrability (Dietz *et al.*, 2007; Lind & Jakobsson, 2001; Lindstrom *et al.*, 2000; Pennycuick, 1975).

The positive relationship between pectoral muscle size and maximal thermogenic capacity confirms previous findings (Cooper, 2002; Marjoniemi & Hohtola, 1999; O'Connor, 1995; Swanson *et al.*, 2013; Vézina *et al.*, 2007). However, this is the first experimental demonstration that muscle size manipulation leads to significant changes in thermogenic capacity and likely cold tolerance (Swanson, 2001). Our findings therefore suggest that flight feather clipping could be used as a tool, not only to manipulate muscle phenotypes in small birds (Lind & Jakobsson, 2001) but also to manipulate heat production capacity. However, it is interesting to note that the relationship we observed between Msum and pectoral muscle score plateaued at intermediate levels of muscle size (figure 3.6). Msum increased by 14-16% when comparing individuals with muscle score 1 to birds with muscle scores 2 and 3 but did not differ significantly between individuals expressing these latter two muscle sizes. This contrasts with previous observations of linear correlations between muscle size and Msum (Swanson *et al.*, 2013; Vézina *et al.*, 2007) and suggests that chickadees developing their flight muscles to a size scored as level 3 would pay an unnecessary maintenance cost since it does not provide additional thermogenic capacity. However, one has to keep in mind that, in this experimental setting, the muscle phenotype

responded to feather clipping and therefore to a loss of wing and tail lift rather than temperature. Thus, the effect of our manipulation on Msum likely resulted from a thermogenic side effect of increased muscle size for locomotor activity (Swanson, 1995; Swanson & Dean, 1999; Vézina *et al.*, 2007). It could be that the relationship between muscles output for locomotion and for shivering is not linear and that, in Black-capped chickadees, muscle sizes corresponding to scores 2 and 3 provided a similar heat production capacity. Alternatively, despite the fact that muscle score does reflect pectoral muscle mass (MP and FV, unpublished data), measuring muscle size using scores may not be precise enough to detect effects on heat production capacity in individuals expressing relatively large pectoral muscles. Linear measurements such as ultrasound scanning (Dietz *et al.*, 1999; Swanson & Merkord, 2013) could improve precision in further studies.

Cold acclimatization is often associated with an increase in hæmatocrit level (deGraw *et al.*, 1979; O'Connor, 1996; Swanson, 1990b) which is considered a response to elevated oxygen demands for thermogenesis (Carey & Morton, 1976; Swanson, 1990b). Our results are consistent with this interpretation. Hæmatocrit levels were not affected by the group, contrarily to what we predicted, but levels were higher after the treatment, between January and March, which includes the coldest months of winter. For a given body mass and pectoral muscle size, birds with higher hæmatocrit levels also expressed a higher Msum and this relationship appeared to plateau when hæmatocrit reached levels around 0.53. Therefore, the pattern of the relationship between hæmatocrit and Msum seemed to parallel that observed between pectoral muscle size and Msum. The parabolic shape in the relationship between Msum and hæmatocrit is consistent with the idea that beyond a certain level, the benefit of increasing oxygen carrying capacity is counterbalanced by the disadvantage of elevated blood viscosity (Schuler *et al.*, 2010; Williams *et al.*, 2012). Therefore, it could be that chickadees have an optimal hæmatocrit level that maximizes their heat production capacity and cold endurance and given that heart mass fluctuates seasonally in small wintering passerines (*e.g.* Zheng et al., 2008), including chickadees (Liknes and Swanson 2011, Petit *et al.* unpublished data), this optimal level is likely to change throughout the year. As far as we know, this is the first demonstration of the relationship between maximal thermogenic capacity and hæmatocrit in birds. However, this link remains correlative and experimental manipulations of hæmatocrit levels (Williams *et al.*, 2012) should be conducted to confirm its role in supporting thermogenesis.

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CHAPITRE IV

Comment la flexibilité de la composition corporelle reflète-t-elle les variations de la performance métabolique chez un petit passereau hivernant sous les hautes latitudes ?

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How does flexibility in body composition relate to seasonal changes in metabolic performance in a small passerine wintering at northern latitudes?

Magali Petit[1,2*], Agnès Lewden[1], François Vézina[1,2]

[1]Département de biologie, chimie et géographie, Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski (QC), G5L 3A1, Canada. Groupe de recherche sur les environnements nordiques BORÉAS, Rimouski (QC), Canada. Centre d'Études Nordiques, Québec (QC), Canada. [2]Centre de la Science de la Biodiversité du Québec, Montréal (QC), Canada.

* Corresponding author. E-mail: magali.petit@uqar.ca

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Contributions - Conception de l'expérience: MP, FV ; Réalisation de l'expérience: MP, AL; Analyse des données: MP ; Financement: FV ; Rédaction : MP, FV.

4.1 Résumé

Les espèces aviaires de petite taille résidant sous les hautes latitudes expriment généralement une augmentation de leur métabolisme de base (BMR) et de leur capacité thermogénique maximale (Msum). Ces ajustements sont communément interprétés comme reflétant des modifications de la composition corporelle, avec l'augmentation de la taille des organes digestifs et excréteurs menant à l'augmentation hivernale du BMR et le développement des muscles squelettiques conduisant à la hausse du Msum. En utilisant la mésange à tête noire (*Poecile atricapillus*) comme espèce modèle, nous avons étudié les changements saisonniers de la composition corporelle et nous avons testé le lien entre la variation de masse des organes et les ajustements du BMR et du Msum. Nos résultats ont confirmé l'augmentation attendue de la masse des muscles et des organes cardiopulmonaires (cœur+poumons) pendant l'hiver et ont montré que 64% de la variation annuelle du Msum était expliqué par les changements de masse de ces tissus. A l'opposé, nous n'avons trouvé que peu d'effet de la masse des organes digestifs (gésier+intestins) sur la variation saisonnière du BMR. A la place, nous avons mis en évidence que le BMR était principalement influencé par la masse des muscles squelettiques et des organes excréteurs (foie+reins), allant jusqu'à expliquer 35% de la variabilité du BMR.

Mots-clefs: métabolisme ; BMR ; Msum ; acclimatation hivernale ; organes.

4.2 Abstract

Small avian species wintering at northern latitudes typically show increases in basal metabolic rate (BMR) and maximal thermogenic capacity (Msum). Those are widely assumed to reflect changes in body composition, with enlargement of digestive and excretory organs resulting in elevated winter BMR and larger body muscles driving the increase in Msum. Using free-living Black-capped chickadees (*Poecile atricapillus*) as our model species, we investigated seasonal changes in body composition and tested for relationships between mass variations of body organs and variability of both BMR and Msum. Our results confirmed the expected winter increase in mass of body muscles and cardiopulmonary organs (heart+lungs) and showed that 64% of the observed Msum variations throughout the year were explained by changes in these organs. In contrast, we found little support for an effect of the digestive organs (gizzard+intestines) on BMR seasonal changes. Instead, this variable was mainly influenced by variations in mass of body muscles and excretory organs (liver+kidney), explaining up to 35% of its variability.

Keywords: metabolic rate; BMR; Msum; winter acclimatization; organs.

4.3 Introduction

Small bird species wintering at northern latitudes routinely face energetic challenges owing to cold ambient temperatures (Cooper & Swanson, 1994; Liknes & Swanson, 1996) and restricted access to food (McNamara & Buchanan, 2005; McNamara *et al.*, 1990; Swanson, 2010). This leads to an array of behavioural and physiological adjustments to improve energy acquisition and thermogenic capacity (Cooper & Swanson, 1994; Swanson, 1991a; Swanson & Thomas, 2007; Vézina *et al.*, 2011). In parallel to these changes, adjustments in metabolic performance are often visible with birds typically expressing higher levels of basal metabolic rate (BMR, seen as the body's physiological maintenance cost) and summit metabolic rate (Msum, an indicator of cold tolerance) in winter relative to summer (Cooper & Swanson, 1994; Swanson, 1991a; Vézina *et al.*, 2006; Zheng *et al.*, 2008).

Given the general interpretation that variations in metabolic parameters, such as BMR, reflect changes in size and activity of body components (Burness *et al.*, 1998; Chappell *et al.*, 1999; Daan *et al.*, 1990; Piersma & Van Gils, 2010; Weber & Piersma, 1996), BMR variations in response to cold are often perceived as the result of body remodelling (Petit *et al.*, 2013; Swanson, 2010; Vézina *et al.*, 2006). For example, Williams and Tieleman (2000) showed that captive cold-acclimated hoopoe larks (*Alaemon alaudipes*) increased their BMR along with the mass of their stomach, intestines, liver and kidneys while Maldonado *et al* (2009) observed a concomitant increase in BMR and the masses of the liver, kidneys and heart in captive cold-acclimated rufous-collared sparrows (*Zonotrichia capensis*). Similar results have also been observed in free-living species when comparing summer and winter phenotypes. Indeed, in Eurasian tree sparrows (*Passer montanus*), Zheng *et al* (2008) found a higher BMR and heavier intestines, liver and heart in individuals captured in winter compared to those captured in summer. A similar finding was obtained by Liu and Li (2006) in the same species where the elevated winter BMR was shown, in independent correlation analyses, to be positively related to the mass of the gizzard, intestines, liver and heart. Although there are differences in which particular organs covary with BMR, these observations suggest a predominant contribution of the digestive organs and heart to the winter increase in physiological maintenance costs (Swanson, 2010).

Seasonal and experimental changes in Msum have also been interpreted in terms of body remodelling. Msum is the maximal shivering heat production capacity (Cooper & Swanson, 1994; Swanson, 1991a; Vézina *et al.*, 2006) and studies highlighted parallel increases in thermogenic capacity and pectoral muscle size in free-living (Cooper, 2002; O'Connor, 1995; Swanson *et al.*, 2013) as well as in captive birds (Vézina *et al.*, 2011; Vézina *et al.*, 2007). Consequently, it is commonly assumed that variations in Msum reflect changes in the size of skeletal muscles (Cooper, 2002; Saarela & Hohtola, 2003; Swanson *et al.*, 2013; Vézina *et al.*, 2007).

We recently showed in free-living Black-capped chickadees (*Poecile atricapillus*) that metabolic performance varied within the winter season (Petit *et al.*, 2013). In this study, we found that BMR remained at its summer level in autumn until November and then increased to reach a peak 6% higher in February while Msum had already achieved 20% of its inter-seasonal change by October, attaining in February a level 36% higher than the summer level measured in August. This within-winter variation in metabolic performance suggests that the size and activity of the organ machinery responsible for changes in metabolic rates should also vary throughout winter. However, the uncoupling in the timing of seasonal changes in BMR and Msum implies that internal organs and skeletal muscles may follow dissimilar paths through time in response to parallel but relatively independent constraints (Petit *et al.*, 2013).

This study follows up on previous work (Petit *et al.*, 2013) and investigates seasonal changes in body composition and metabolic performance of free-living black capped chickadees wintering in eastern Canada. Our objectives were (1) to characterize the patterns of organ mass variation throughout the year with a focus on the winter season; (2) to determine the contribution of these organs to BMR and Msum variations and (3) to determine if the effect of specific organs on metabolic rate variations were constant over the year. We expected that chickadees would cope with the winter increase in thermoregulation and energy requirements by enlarging exercise (*e.g.* muscles, heart, lungs) (Liknes & Swanson, 2011b) as well as digestive and excretory organs (*e.g.* intestines, liver, stomach, kidneys) (Williams & Tieleman, 2000) and that this would consequently explain the increase in metabolic performance associated with the peak of cold. More precisely, based on observations by others, we expected a predominant role of the digestive (*e.g.* intestines) and excretory (*e.g.* kidneys) organs in explaining BMR variations (Liu

& Li, 2006; Maldonado *et al.*, 2009; Zheng *et al.*, 2008), while Msum would be mainly influenced by exercise organs such as breast muscles and heart (Chappell *et al.*, 1999; Cooper, 2002; Liknes & Swanson, 2011b; O'Connor, 1995).

4.4 Materials and methods

4.4.1 Capture and handling

We captured 79 free-living Black-capped chickadees (42 males and 37 females) within the Forêt d'Enseignement et de Recherche Macpès, Québec, Canada (48°18' N, 68°31' W) from November 12th to 22nd 2010 (fall, mean temperature on site: -0.7 ± 5.2°C, n = 20), February 3rd to 15th 2011 (midwinter, mean temperature on site: -11.9 ± 5.1°C, n = 28), March 23rd to 31st 2011 (end of winter, mean temperature on site: -3.9 ± 4.3°C, n = 20) and August 11th to 21st 2011 (summer, mean temperature on site: non available, mean temperature recorded by the closest weather station 19 km away: 19.1 ± 1.9°C, n = 11). To attract chickadees and facilitate capture, 16 feeding stations were set up with an average distance between stations of 1.9 km (Lewden *et al.*, 2012; Mandin & Vézina, 2012). Feeders were regularly filled with black oil sunflower seeds. On capture days, feeders were removed and homemade potter traps (15 cm x 15 cm x 15 cm) baited with black oil sunflower seeds were used. All birds were caught between 08:00 and 13:00 and handled in less than ten minutes following capture.

We weighed birds and measured the lengths of their beak, head plus beak, tarsus, tail and wing (Pyle, 1997). Following these measurements and depending on capture success, up to four birds per day were brought to the field station for metabolic measurements. All bird manipulations were approved by the animal care committee of the Université du Québec à Rimouski and have been conducted under scientific and banding permits from Environment Canada - Canadian Wildlife Service.

4.4.2 Respirometry

Once at the field station, birds were maintained in separate cages (39 cm x 43 cm x 31 cm) supplied with food (sunflower seeds) and water *ad libitum* until measurements. Cages were

kept in a room receiving natural light and maintained quiet. At around 13:00, we began Msum trials by measuring two birds in parallel using the instruments and protocol described by Lewden *et al.* (2012) and Petit *et al* (2013). This was followed by a second trial on the remaining two birds, which began before 15:00. Measurements were carried out as follows. Birds were first weighed (± 0.1 g) and body temperature was measured with a thermocouple thermometer (Omega model HH-25KC, NIST-traceable, Omega, Montréal, QC, Canada) using a copper-constantan thermocouple inserted into the cloacae approximately 10 mm deep. Then, birds were put in a stainless steel metabolic chamber fitted with a perch and were exposed to dry, CO_2-free helox gas (21% oxygen, 79% helium) using an average flow rate of 1109 $ml.min^{-1}$. We recorded oxygen consumption of each bird using a sliding cold exposure protocol (Swanson *et al.*, 1996) with a decrease in ambient temperature of 3°C every 20 minutes, starting at 3°C in the fall, 0°C in winter and 6°C in summer. We ended the trials when birds became hypothermic which was easily identifiable in real time as a steady decline in oxygen consumption for several minutes. Body temperature was measured again immediately after taking the birds out of their chamber. We assumed a bird reached its Msum when body temperature after a trial was ≤ 38°C (Cooper & Gessaman, 2005) (mean body temperature after Msum measurement = 34.1 ± 0.3°C). Data from individuals showing a body temperature above this threshold were discarded. Birds were weighed again after measurements and the average body mass was used for the Msum analysis. Birds were then brought back to their cage with food and water *ad libitum* until BMR measurement, starting at 19:00.

Each day, all four birds had their BMR measured simultaneously overnight (from 19:00 to 06:00). Individuals were maintained at 30°C throughout the trial (within the thermoneutral zone for this species, Rising & Hudson, 1974) and received a constant air flow of 470 $ml.min^{-1}$. Birds were weighed before and after measurements and average mass was used in BMR analyses. Oxygen analyzers (FoxBox, Sable Systems, Las Vegas, NV, USA) were adjusted each day using CO_2-free dry air. Mass flow valves (Sierra Instruments, Side-Trak® Model 840, Monterey, CA, USA) were calibrated for air and helox using a bubble-O-meter (Dublin, OH, USA). Metabolic rate calculations were done with ExpeData software, v1.2.6 (Sable Systems, Las Vegas, NV, USA). Msum and BMR were calculated according to Lighton's equation 10.1 (Lighton, 2008) and based respectively on the highest and lowest

averaged 10 minutes of oxygen consumption per measurement sequence. Oxygen content in the excurrent flow was measured every 5 seconds and every 20 seconds for Msum and BMR measurements, respectively. The instantaneous measurement technique (Bartholomew *et al.*, 1981) was used for Msum while BMR was calculated using the steady state approach. Since wintering birds use mainly lipids as substrate for shivering (Swanson, 2010), we estimated energy consumption using a constant equivalent of 19.8 $kJ.L^{-1}$ O_2 and converted to Watts (Gessaman & Nagy, 1988). After BMR measurements, birds were put back in their cage for feeding and rehydratation until sacrifice for organ data 2 hours later.

4.4.3 Organ mass

Birds were anaesthetized by an injection of a ketamin-xylasine mixture (0.005 $ml.g^{-1}$) in the right pectoral muscle and sacrificed by exsanguination. Breast feathers were plucked to create a ventral opening from the cloacae to the beak and birds were then dissected. Biopsies of the left *pectoralis* muscle (0.084 ± 0.002 g), liver (0.067 ± 0.002 g), heart (0.079 ± 0.003 g), empty intestines (0.020 ± 0.001 g), left kidney (0.003 ± 0.001 g) and ventral muscles of the left leg (0.056 ± 0.001 g) were weighed and kept in liquid nitrogen for further analyses (not shown here). Gizzard and the remaining empty intestines were also collected and frozen at -20°C. The rest of the feathers were then plucked and birds were stored at -20°C until we could complete dissections in the laboratory.

At the laboratory, we collected and weighed the wet mass (± 0.001 g) of the right and left *pectoralis* and *supracoracoideus* muscles, the remaining muscles of the right and left legs, the *proventriculus*, the lungs, the brain, the skin, the remaining parts of the heart, the liver and kidneys as well as the carcass. Then, samples were freeze-dried (FreeZone 2.5, Labconco, Kansas city, KS, USA) for 2 days to obtain constant dry mass of tissues (± 0.001 g). Following drying, lipids were extracted from samples with a soxhlet apparatus using petroleum ether and re-dried overnight to obtain lean dry masses of organs and carcass (± 0.001 g). Carcasses were then burned in a muffle furnace at 500°C for 10-12 hours to measure ash mass and to calculate ash free lean dry mass of carcasses. This is mainly the lean dry mass of the rest of skeletal muscles (thereafter called "skeletal muscles"). The mass of symmetrical paired organs (*e.g.* pectoral muscles) was calculated by summing the mass of both sides while the mass of biopsied

organs was calculated by adding fresh biopsy masses to the remaining fresh mass of the organs and recalculating lean dry mass according to the proportion of water and lipids contained in the tissues. We did not record the mass of the reproductive organs neither the lean dry mass of the spleen, pancreas and *proventriculus* because lean dry samples were too small to be weighed accurately. Technical difficulties associated with manipulating very small dried organs also led to the loss of some samples. In total, we obtained data for all organs in 64 birds. Each bird was sexed by inspection of reproductive organs during dissections.

4.4.4 Statistical analysis

4.4.4.1 Seasonal variation and sex effects on body mass and metabolic performance

Body mass varies with individual body size. To study changes in body mass throughout the year we therefore had to consider this effect. We used a measure of structural body size as covariate to analyse change in size-independent body mass. The structural body size (thereafter called "size") was calculated from a principal component analysis on morphological data (length of head plus beak, wing and tarsus) (Rising & Somers, 1989). Then, we used a general linear model (GLM) testing for the effects of "period" (fall, midwinter, end of winter and summer), "sex" (male and female), the interaction term "sex*period", the variable "relative time of capture" (time since sunrise / day length, hereafter "time of capture") to consider the effects of daily fattening (Mandin & Vézina, 2012) and "size" as covariate to investigate how size-independent body mass varied through time. We did the same analysis to study the variation in lean dry body mass.

Metabolic parameters are influenced by the amount of metabolically active tissues which forms most of body mass. Therefore, studies on metabolic rate variation should consider the body mass effect. However, Black-capped chickadees are fattening on a daily basis during winter (Mandin & Vézina, 2012) and most of the body mass variation during that period is thought to result from the accumulation of fat tissues known to have very low metabolic activity (Chaplin, 1974; Scott & Evans, 1992). Statistically controlling metabolic rate for body mass can lead to underestimations when body mass contains a large proportion of fat tissue (Petit *et al.*, 2010). We therefore first studied the variation of whole BMR and whole Msum throughout the

year using a GLM testing for effect of the period, sex and the interaction term sex*period. Then, to control for the effect of metabolically active tissues, we investigated the variation in mass-independent metabolic rates by including lean dry mass as covariate in the models.

4.4.4.2 Seasonal variation in organ mass

We used a GLM testing for the effects of period, sex and the interaction term period*sex to investigate how body composition varied through time. Since the mass of organs may vary with individual body size, we also included size as covariate in these analyses. We also considered the effect of storage time in the freezer. However, this effect was not significant and we did not include this variable in further models.

4.4.4.3 Relationship between metabolic performance and organ mass

Our statistical approach (see below) was limited by our sample size in the number of variables that can be entered in models. For this reason and to avoid collinearity between organ variables, we combined the mass of organs according to their function and seasonal variation patterns (figure 4.1). We used Pearson correlations to test for collinearity between these organ groups and since all coefficients were < 0.60 (table 4.1), we included all organ groups in further analyses. Combined organs were gizzard and intestines (thereafter called "digestive organs"); heart and lungs (thereafter called "cardiopulmonary organs"); skeletal, leg, *pectoralis* and *supracoracoideus* muscles (thereafter called "muscles"); kidneys and liver (thereafter called "excretory organs"). The brain and skin were not included in any group and were used as is.

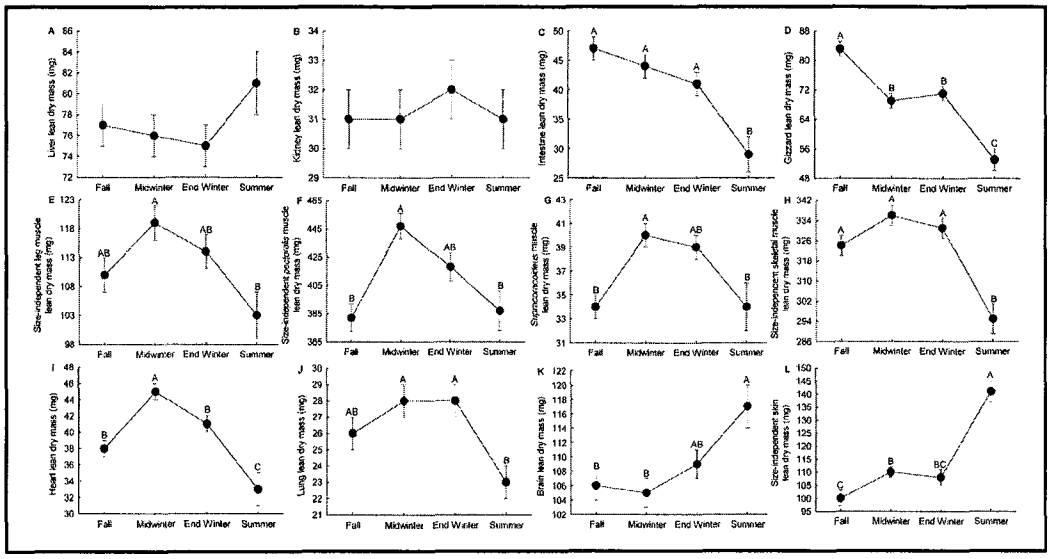

Figure 4.1 Seasonal variation in body composition throughout the year. Data are least square mean of lean dry mass of A) liver, B) kidneys, C) intestines, D) gizzard, E) size-independent leg muscles, F) size-independent *pectoralis* muscles, G) *supracoracoideus* muscles, H) size-independent skeletal muscles, I) heart, J) lungs, K) brain and L) size-independent skin. Least square means were obtained from a GLM including period, sex, period*sex as independent variables and body size as covariate. Different letters represent significant difference between periods.

Table 4.1 Matrix of correlation showing all comparisons between lean dry masses of organ groups.

	Muscles	Heart+Lungs	Liver+Kidney	Intestine+Gizzard	Skin
Heart+Lungs	0.55***				
Liver+Kidneys	0.31**	0.38***			
Intestine+Gizzard	0.13	0.26*	0.08		
Skin	0.25*	-0.10	0.28*	-0.35***	
Brain	0.38***	-0.07	0.22	-0.05	0.39***

*** $p < 0.0001$; ** $p < 0.001$; * $p < 0.05$

Since body mass contains the mass of all organs, statistically controlling metabolic rate for body mass in analyses testing for relationships between body components and metabolic rate would be conceptually biased. We therefore first tested for the effects of sex and body size, rather than body mass, on BMR and Msum using a GLM. This approach considers potential

inter-individual variation in metabolic performance due to sex and body size without removing the effect of organ mass on metabolic rates. BMR and Msum variations were not significantly affected by sex and Msum was not related to size. However, the effect of body size on BMR approached significance ($p = 0.06$). We therefore chose to use a conservative approach and extracted size residuals of BMR to consider this weak effect. We tested for the effects of sex and body size on organ masses with the same approach and extracted residuals for further analysis when the effects were significant. This was found to be the case for muscles, cardiopulmonary organs, excretory organs, brain and skin but not for digestive organs. The following step was to test for relationships between residual BMR or whole Msum and residuals of organ mass (except for digestive organs). We did this using data for the whole year and then for each period independently. We used a model selection approach based on second order Aikaike information criteria (AICc) to highlight the best model explaining variations in metabolic performance. Standardized coefficients (β) were used to order organs according to their relative influence on the dependent variable.

In GLM analyses, significant differences between periods were examined using Tukey tests. For all analyses, model residuals were tested for normality using one sample Kolmogorov-Smirnov test. Data are presented as least square means ± s.e.m. Model selection were performed in R using the MuMIn package (Barton, 2013).

4.5 Results

4.5.1 Seasonal variation in body mass and metabolic performance

Size-independent body mass (size: $F_{1,72} = 8.5$, $p < 0.01$) varied over the year (period: $F_{3,72} = 7.3$, $p < 0.001$). Body mass increased by 7.0% between fall and midwinter (Tukey: $p < 0.0001$), decreased by 4.0% (Tukey: $p < 0.05$) by the end of winter and remained constant until summer (figure 4.2). Size-independent body mass was positively related to time of capture ($F_{1,72} = 5.5$, $p < 0.05$) and was also dependent on sexes ($F_{1,72} = 35.7$, $p < 0.0001$) with males being on average 7.8% heavier than females (males: 11.6 ± 0.1 g; females: 10.8 ± 0.1 g,) and this effect did not change between the periods (period*sex: $p = 0.4$). Size-independent lean dry mass (size: $F_{1,73} = 11.5$, $p < 0.01$) was not affected by time of capture ($p = 0.2$) or the interaction term

period*sex ($p = 0.2$) but varied with period ($F_{3,73} = 4.6$, $p < 0.01$) and sex ($F_{1,73} = 37.2$, $p < 0.0001$). Lean dry mass increased by 5.7% between fall and midwinter (Tukey: $p < 0.05$) then decreased by 7.6% between midwinter and summer (Tukey: $p < 0.01$) (figure 4.2). When controlling for size, males (1.46 ± 0.02 g) had on average 10.3% more lean dry mass than females (1.32 ± 0.02 g).

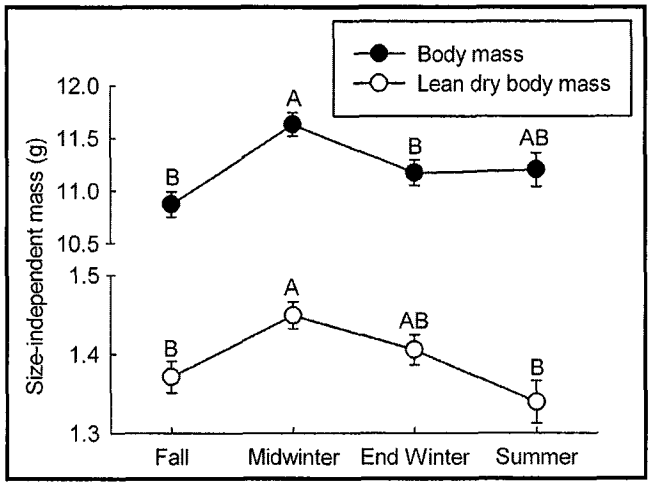

Figure 4.2 Seasonal variation in body mass and lean dry body mass throughout the year. Data are least square mean obtained from a GLM including period, sex, relative time of capture and period*sex as independent variables as well as body size as covariate. Different letters represent significant difference between periods.

Whole BMR did not vary with sex ($p = 0.1$) or the interaction term period*sex ($p = 0.06$) but varied with period ($F_{3,73} = 3.2$, $p < 0.05$). BMR increased, although not significantly in *post-hoc* analyses, by 4.1% from fall to midwinter (Tukey: $p = 0.3$) and then decreased significantly by 5.8% by the end of winter (Tukey: $p < 0.05$) to remain stable until summer (Tukey: $p = 1.0$) (figure 4.3a). Mass-independent BMR (lean dry mass: $F_{1,74} = 46.2$, $p < 0.0001$) was only related to sex ($F_{1,74} = 8.5$, $p < 0.01$) with females (0.265 ± 0.003 W) expressing on average a BMR 5.6% higher than males (0.251 ± 0.003 W) (effect of period and period* sex $p > 0.1$ in both cases).

Whole Msum was not influenced by the interaction term period*sex ($p = 0.6$) but varied with sexes ($F_{1, 64} = 4.9$, $p < 0.05$) and periods ($F_{3, 64} = 14.0$, $p < 0.0001$). Males expressed a Msum (1.481 ± 0.028 W) on average 6.6% higher than females (1.389 ± 0.031 W). Average Msum increased by 14.4% between fall and midwinter (Tukey: $p < 0.01$), stayed constant until the end of winter (Tukey $p = 0.9$) and then decreased by 22.2% in summer (Tukey $p < 0.0001$). Mass-independent Msum (lean dry mass: $F_{1, 64} = 23.5$, $p < 0.0001$) was not influenced by the interaction period*sex ($p = 1.0$) or sex ($p = 0.3$) but also varied with period ($F_{3, 64} = 15.1$, $p < 0.0001$). Mass-independent Msum reached a peak in winter (+12.5% between fall and midwinter, Tukey: $p < 0.01$), stayed constant until the end of winter (Tukey: $p = 1.0$) and declined by 19.5% between the end of winter and summer (Tukey: $p < 0.0001$) (figure 4.3b).

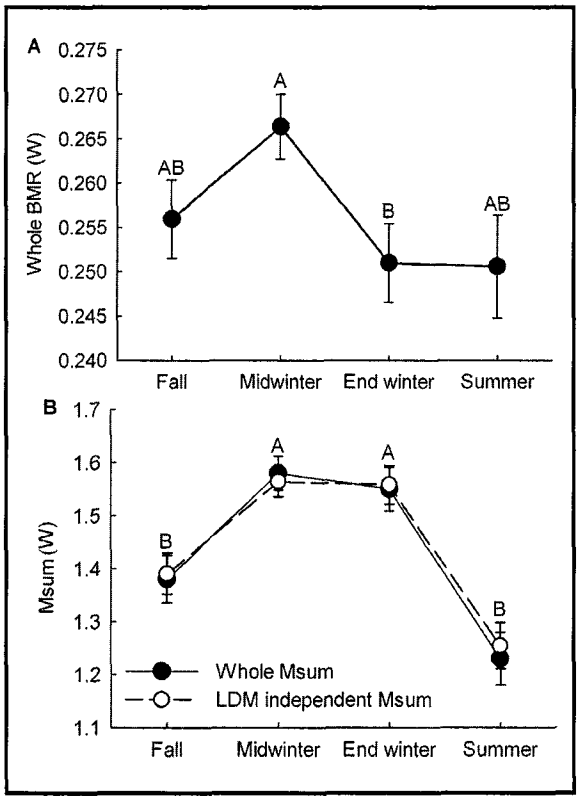

Figure 4.3 Metabolic variations throughout the year. Least square mean of A) BMR and B) Msum were obtained from a GLM including period, sex and period*sex as independent variables. Different letters represent significant difference between periods. LDM : lean dry mass.

4.5.2 Seasonal variation in lean dry organ mass

The lean dry mass of digestive organs was influenced by body size ($F_{1, 73} = 4.3$, $p < 0.05$) and periods ($F_{3, 73} = 26.3$, $p < 0.0001$) but not by sex ($p = 0.7$) or the interaction period*sex ($p = 0.6$). Digestive organs showed a declining pattern throughout the year when considering the effect of body size. Indeed, they declined by 13.3% (Tukey: $p < 0.01$) from fall to the end of winter and then decreased by a further 28.8% between the end of winter and summer (Tukey: $p < 0.0001$) (figure 4.4a).

Cardiopulmonary organs were not affected by body size ($p = 0.8$) or the interaction period*sex ($p = 0.2$) but their lean dry mass varied with sex ($F_{1, 72} = 8.9$, $p < 0.01$) and periods ($F_{3, 72} = 12.2$, $p < 0.0001$). Males (68 ± 1 mg) had 9.7% heavier heart+lungs than females (62 ± 1 mg). These organs increased in mass by 14.0% between fall and midwinter (Tukey: $p < 0.01$), then started to decline, although non-significantly ($p = 0.2$) at the end of winter and decreased by 17.6% between the end of winter and summer (Tukey: $p < 0.001$) (figure 4.4b).

Variations in lean dry muscle mass were affected by body size ($F_{1, 71} = 14.0$, $p < 0.001$), sex ($F_{1, 71} = 32.7$, $p < 0.0001$) and period ($F_{3, 71} = 11.5$, $p < 0.0001$) but not by the interaction period*sex ($p = 0.1$). For a given structural size, males (926 ± 11 mg) had muscles 11.2% heavier than females (833 ± 11 mg). Muscle mass increased by 10.7% between fall and midwinter (Tukey: $p < 0.0001$). It then tended to decline non-significantly toward the end of winter ($p = 0.3$) and decreased by 9.8% from the end of winter to summer (Tukey: $p < 0.01$), to a level indistinguishable from that recorded in the fall (Tukey: $p = 0.5$) (figure 4.4c).

The lean dry mass of excretory organs varied independently from the effects of body size ($p = 0.5$), periods ($p = 0.6$) and the interaction term period*sex ($p = 0.3$). It was, however, affected by sex ($F_{1, 74} = 6.4$, $p < 0.05$) with males (111 ± 2 mg) having their kidneys+liver 6.5% heavier than females (104 ± 2 mg).

Variations in brain mass were independent from body size ($p = 0.3$) and the interaction term period*sex ($p = 0.7$) but were related to variation in these latter variables taken independently (sex: $F_{1, 71} = 17.0$, $p < 0.0001$, period: $F_{3, 71} = 3.6$, $p < 0.05$). Males (115 ± 2 mg) had a lean dry brain mass 10.6% heavier than that of females (104 ± 2 g) and average brain mass was at its highest in summer with a value 10.4% and 10.5% higher than in the fall (Tukey: $p < 0.05$) and midwinter (Tukey: $p < 0.05$) respectively (figure 4.4d).

Lean dry skin mass varied with body size ($F_{1, 70} = 5.4$, $p < 0.05$), sex ($F_{1, 70} = 6.2$, $p < 0.05$) and period ($F_{3, 70} = 29.5$, $p < 0.0001$) but not with the interaction period*sex ($p = 0.4$). Controlling for size, skin mass in males (119 ± 2 mg) was 7.2% heavier on average than in females (111 ± 2 mg). Lean dry mass of the skin increased by 10.0% between fall and midwinter (Tukey: $p < 0.05$), remained stable until the end of winter (Tukey: $p = 0.9$) and then increased by a further 30.6% between end of winter and summer (Tukey: $p < 0.0001$) (figure 4.4e).

Fat content was not dependent on size ($p = 0.1$), sex ($p = 0.4$) or the interaction sex by period ($p = 0.1$) but changed with the period ($F_{3,74} = 4.3$, $p < 0.01$). It remained constant in the fall and midwinter and declined progressively to reach a level 25.8% lower in summer (Tukey: $p < 0.01$) (figure 4.4f).

We found no significant period by sex interaction ($p = 0.5$) on water content but body water varied with size ($F_{1,72} = 13.8$, $p < 0.001$), sex ($F_{1,72} = 25.1$, $p < 0.0001$) and period ($F_{3,72} = 3.3$, $p < 0.05$). Controlling for size, the male's body (5170 ± 70 mg) contained on average 6.3% more water than that of females (4860 ± 70 mg). Water content increased by 8.4% between the fall and midwinter (Tukey: $p < 0.01$) and then decreased by 6.4% by the end of winter (Tukey: $p < 0.05$) to stay constant until summer (figure 4.4g).

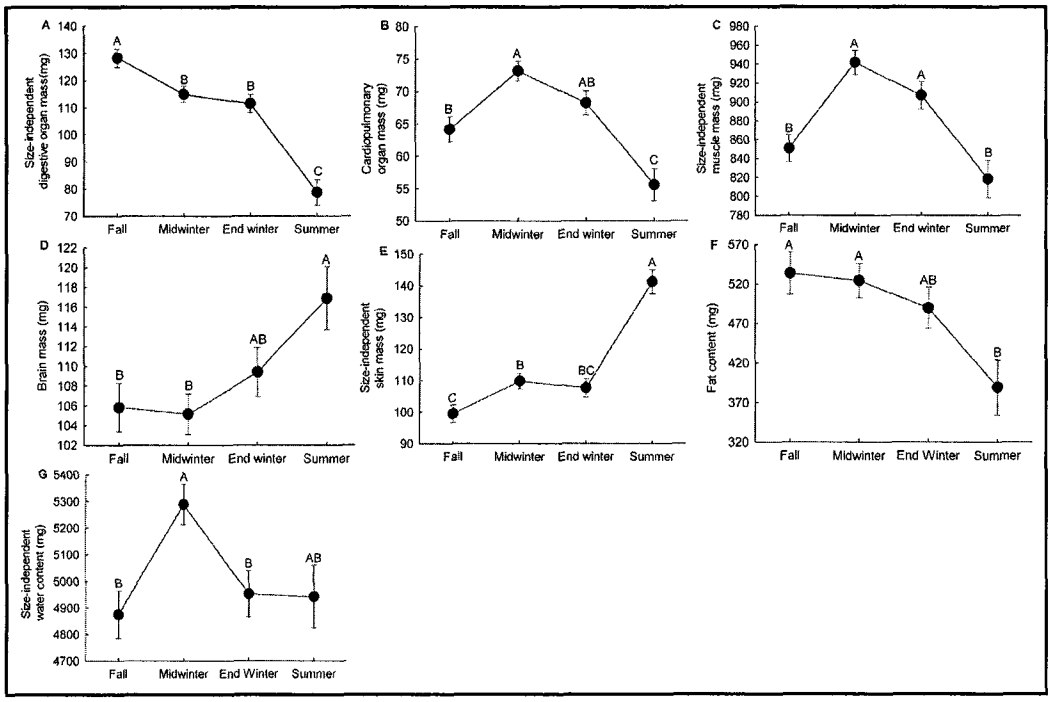

Figure 4.4 Seasonal variation in organ groups throughout the year. Least square mean of A) size-independent digestive organ, B) cardiopulmonary organ, C) size-independent muscle, D) brain and E) size-independent skin lean dry masses as well as of F) fat and G) size-independent water content. Least square means were obtained from a GLM including period, sex, period*sex as independent variables (and size as covariate for digestive organs, muscles, skin and water content). Different letters represent significant difference between periods.

4.5.3 Relationship between metabolic performance and body composition

4.5.3.1 BMR

Considering data for the whole year, the best model ($F_{2, 61}$ = 16.0 $p < 0.0001$), explaining 32% of the variation in BMR, included the residual mass of muscles ($F_{1, 61} = 27.6$, $p < 0.0001$, $\beta = 0.52$) and residual mass of the excretory organs ($F_{1, 61} = 4.5$, $p < 0.05$, $\beta = 0.22$). During the fall, variations in BMR were best explained (r^2_{adj} = 0.30) by a model ($F_{1, 14}$= 7.5, $p < 0.05$) including only residual muscle mass ($F_{1, 14}$= 7.5, $p < 0.05$, $\beta = 0.59$) while in midwinter, 43% ($F_{3, 19} = 6.5$, $p < 0.01$) of the variation in BMR was explained by a combination of the residual mass of excretory organs ($F_{1, 19}$ = 13.0, $p < 0.01$, $\beta = 0.60$), brain ($F_{1, 19} = 4.8$, $p < 0.05$, $\beta = 0.43$) and the lean dry mass of digestive organs (p = 0.2). Analyses for the end of winter revealed that the best model ($F_{1, 12}$ = 8.1, $p < 0.05$, r^2_{adj} = 0.35) explaining BMR variations included only residual muscle mass ($F_{1, 12}$ = 8.1, $p < 0.05$, $\beta = 0.63$) while during the summer, the best model was the null model (figure 4.5).

4.5.3.2 Msum

Model selection showed that over the whole year, Msum variations were best explained ($F_{3, 2} = 32.9$, $p < 0.0001$, r^2_{adj} = 0.64) by a combination of residual lean dry muscle mass ($F_{1, 52} = 33.6$, $p < 0.0001$, $\beta = 0.60$), residual mass of cardiopulmonary organs ($F_{1, 52} = 62.4$, $p < 0.0001$, $\beta = 31$) and residual excretory organ mass (p = 0.1). In the fall, model selection produced a model ($F_{1, 9} = 10.3$, $p < 0.05$, $r^2_{adj} = 0.48$) that included the lean dry mass of digestive organs ($F_{1, 9}$ = 10.3, $p < 0.05$, β = 0.73) while for midwinter, the best model ($F_{1, 20}$ = 28.2, $p < 0.0001$), explaining 56% of the variation in Msum, included only the residual mass of muscles ($F_{1, 20} = 28.2$, $p < 0.0001$, β = 0.76). Similarly, at the end of winter, the best model ($F_{1, 10}$= 10.3, $p < 0.01$) explained 46% of Msum variation and included only residual muscle mass ($F_{1, 10} = 10.3$, $p < 0.01$, $\beta = 0.71$). Finally, in the summer, no organs were found to explain any significant part of variations in Msum (figure 4.5).

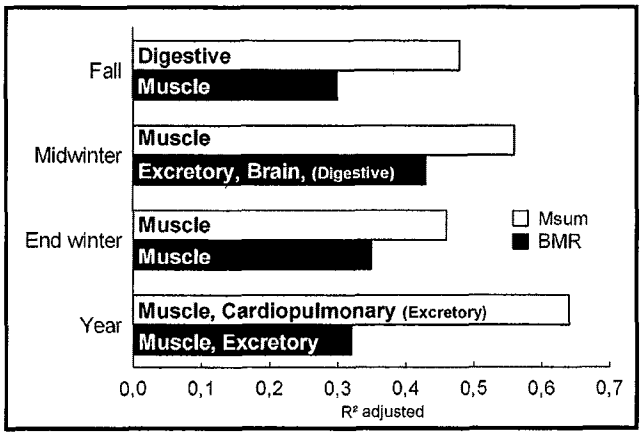

Figure 4.5 Contribution of body components to metabolic performance throughout the year. Bars represent the r^2 adjusted from the best model explaining size-independent BMR or whole Msum for each period (see methods). Organs contributing to the models are given in bars. Organs between ( ) were retained in the models but were not significant. Summer is not represented since BMR and Msum were not found to relate to body components during this period.

4.6 Discussion

In this study, we predicted that chickadees would express higher metabolic performance in midwinter, at the peak of cold, and that this would be mainly explained by enlargements in exercise, digestive and excretory organs. More specifically, we expected a significant contribution of the digestive and excretory organs in BMR variations while changes in Msum would mainly result from variations in the size of exercise organs such as the muscles. Our results only partially supported these predictions. As expected, thermogenic capacity was higher and muscles were larger at midwinter than at any other periods and muscles explained most of the variation in Msum at that time. However, seasonal changes in BMR were minimal (4-6%) and non-significant when considering the effect of lean dry body mass. Visceral organs showed either no seasonal change (*e.g.* excretory organs) or a decline from fall to summer (*e.g.* digestive organs) and although excretory organs were found to relate

significantly with BMR at midwinter, the effect of the digestive organs on that variable was not significant.

4.6.1 Seasonal variation in body mass and metabolic performance

Black-capped Chickadees, as other passerines (Lehikoinen, 1987), have been shown to express daily variations in body mass during winter, which are interpreted as changes in the size of endogenous lipid reserves (Mandin & Vézina, 2012). Our results are in agreement with this interpretation as we found a significant effect of time of capture on size-independent body mass but not on size-independent lean dry mass. At the seasonal scale, although body fat content remained relatively constant from the fall to the end of winter, we found a midwinter peak in both size-independent body mass and size-independent lean dry body mass (figure 4.2). The variations in lean dry body mass paralleled that observed in muscles and cardiopulmonary organs (compare figures 4.2 and 4.4) and the peak of mass coincided with that of body water content. In contrast with earlier findings suggesting that seasonal winter body mass variations are the sole result of fat accumulation in Black-capped chickadees (Chaplin, 1974), our results suggest that the winter peak in body mass is also resulting from a larger proportion of lean tissues (*e.g.* skeletal muscles) in the body, which are known to contain more water relative to fat (Gerson & Guglielmo, 2011a, 2011b).

As observed in other studies on chickadees and other avian taxa (Cooper & Swanson, 1994; Vézina *et al.*, 2006; Zheng *et al.*, 2008), birds increased their metabolic performance during winter. Whole BMR culminated at midwinter with values on average 6% higher than during summer while whole Msum was on average 29% higher at midwinter relative to summer. These values are consistent with previous observations conducted at the same time in the same population but using a larger sample size. Indeed, Petit *et al* (2013) reported a 6% increase in BMR and a 34% increase in Msum between peak of winter and summer in different birds from the same population. In the present case, however, the seasonal changes in BMR were not significant when considering the effects of lean dry body mass. Therefore, as suggested earlier (Petit *et al.*, 2013), it appears that the increase in heat production capacity associated with cold acclimatization does not require an upregulation of maintenance costs in our population. BMR

may thus be less affected by ambient temperature than Msum (Petit *et al.*, 2013; Swanson *et al.*, 2012).

4.6.2 Variation in organ mass between sexes

When testing for the effects of period, sex and size on organ mass, we found that males had heavier brain, skin as well as heavier muscles, cardio-pulmonary and excretory organs than females while digestive organs did not differ between sexes. These findings are consistent with previous observations of a sex effect on structural body size and body mass in chickadees. In general, males of this species are structurally larger and heavier than females (Desrochers, 1990; Lewden *et al.*, 2012; Mennill *et al.*, 2003) and, accordingly, we found that nearly all organs were heavier in males. This shows that the known sex effect on body mass is not simply due to contrasting fat reserves between sexes but also to differences in the lean components of body mass. Digestive organs did not differ between sexes but these are highly flexible and respond to food intake in birds (Dekinga *et al.*, 2001; van Gils *et al.*, 2005; Zheng *et al.*, 2008). The lack of sex effect on these organs therefore likely results from a stronger influence of individual's daily energy requirements rather than sex differences in the amount of metabolic tissue requiring energy.

4.6.3 Variation in organ mass and relationships with BMR

We predicted that winter acclimatization in chickadees would be accompanied by enlargements in the organs involved in digestion and excretion, as a result of elevated energy demands and daily food intake at the peak of winter, and that variations in the mass of these organs would consistently explain variability in BMR (*i.e.* increased demand hypothesis, Williams & Tieleman, 2000). Our results were only partially consistent with these predictions. Although whole BMR was highest at the peak of winter, the increase was modest (4-6%) and did not appear to vary consistently with changes in visceral organs. Indeed, excretory organs (liver+kidney) remained relatively stable throughout the year, a finding similar to that reported by Liknes and Swanson (2011b) for the same organs in the same species and by Liu and Li (2006) and Zheng *et al.* (2008) for the kidneys in Eurasian tree sparrows. This happened while

the digestive organs (gizzard+intestines) showed a decline in mass by 38% between the fall and summer, a finding also consistent with observations in Eurasian tree sparrows where the intestines were reported to have their highest mass in the fall relative to winter and summer (Liu & Li, 2006; Zheng *et al.*, 2008).

Although it remained stable, the mass of the excretory organs nevertheless explained part of the variations in BMR when pooled data were analysed. It was also found to relate significantly with BMR during midwinter. This therefore suggests a significant influence of the mass of the liver and kidneys on maintenance costs at the peak of winter, a finding that is not surprising given the key role played by these organs in maintaining fuel delivery to tissues and the indirect effects of the kidneys on oxygen delivery. Indeed, in addition to its regulating role in carbohydrate, lipid and protein metabolism, the liver produces bile, which is required for assimilating lipids from the diet. The liver has also been suggested as a potential thermogenic tissue in birds and mammals (Baconnier *et al.*, 1979; Vézina *et al.*, 2006; Villarin *et al.*, 2003; Zheng *et al.*, 2013a). The kidneys are a major site of erythropoietin production in birds (Jacobson *et al.*, 1957) and therefore plays an indirect role in regulating oxygen delivery by blood cells. Under cold conditions, birds are known to increase their food intake (Vézina *et al.*, 2006), thermogenic capacity (Cooper & Swanson, 1994; Swanson, 1991a) and hæmatocrit level (the proportion of red blood cell in total blood) (Petit & Vézina, 2014a; Swanson, 1990b). Therefore, although it is not possible to distinguish the specific contribution of the liver or the kidneys in our study, the effect of the excretory organs on midwinter BMR could be driven by their role in red blood cell production, food processing and heat production.

Model selection identified the digestive organs as contributors to BMR variations during midwinter. However, this effect was weak at best since it was not significant and ranked third in importance after the excretory organs and brain. This finding came as a surprise given the experimental and field observations of enlarged digestive organs in cold acclimated or acclimatized birds that support the increased demand hypothesis (Liknes & Swanson, 2011b; Liu & Li, 2006; Williams & Tieleman, 2000; Zheng *et al.*, 2013a). However, as far as we are aware, current evidences are based on observed parallel changes or simple regressions between digestive organ mass and BMR, with no study presenting multivariate analyses to establish the contribution of these organs in the observed variations in BMR. Although digestive organs were

46% larger at the peak of winter relative to summer in chickadees, our observations suggest that winter BMR varies relatively independently from changes in the mass of digestive organs in this species. The lack of relationship between body composition and BMR during the summer was also a surprising result but, although we cannot rule out that a decline in tissue metabolic intensity at that time (Zheng *et al.*, 2008; Zheng *et al.*, 2013b) may uncouple relationships between organ mass and BMR, this could also be due to our small sample size for this period (n = 11).

An organ variable that was found to consistently affect BMR throughout the year was lean dry muscle mass. Indeed, body muscles explained up to 35% of BMR variations in three out of five sets of analyses and were selected as the first variable of importance when using pooled data for all periods. Despite the current interpretation of BMR being a variable mainly reflecting variations in the mass and activity of organs found in the internal cavity (Burness *et al.*, 1998; Liu & Li, 2006; Piersma *et al.*, 1996; Piersma & Van Gils, 2010), our findings corroborate previous studies (Chappell *et al.*, 1999; Konarzewski *et al.*, 2000) where a significant relationship was found between BMR and muscle mass. Because BMR reflects the energy consumption of all lean tissues in a resting animal and since the lean dry mass of muscles represented 64% of total lean dry body mass in our birds, it appears that the main contributing tissue to BMR variation in chickadees was skeletal muscles. Muscles consume little energy at rest relative to their maximal capacity (Scott & Evans, 1992). However, because they represent a large part of total lean tissue mass, variations in their size may inevitably have a strong influence on BMR.

4.6.4 Variation in organ mass and relationships with Msum

We predicted that winter acclimatization in chickadees would be associated with an enlargement of the exercise organs to improve heat production capacity and cold endurance and that the variation in mass of these organs (muscles and cardiopulmonary) would explain most of the variability in Msum. As expected, Black-capped chickadees expressed their highest level of Msum at midwinter (+12-14% relative to fall) and Msum showed a seasonal pattern remarkably well paralleled by the enlargement of the muscles and cardioplumonary organs (+11% and +14% between fall and midwinter, respectively, compare also figures 4.3b with 4.4b and 4.4c).

The observed increase in the mass of exercise organs is consistent with previous studies on wintering passerines where larger pectoral muscles and heart were found in winter relative to summer (Liknes & Swanson, 2011b; O'Connor, 1995; Swanson, 1991b).

As a result of the concomitant increase in Msum and exercise organs, we found that body muscles significantly explained Msum variation in three out of five sets of analyses. This supports the widely accepted interpretation that maximal thermogenic capacity in birds depends mainly on shivering capacity and therefore on muscle mass (Cooper, 2002; Petit & Vézina, 2014a; Saarela & Hohtola, 2003; Swanson *et al.*, 2013; Vézina *et al.*, 2007). Over the complete year, in addition to the effect of muscles, we also detected a significant effect of the cardiopulmonary organs, as we expected, while the excretory organs (*i.e.* liver+kidney) were selected as contributor to Msum variations, although being non significant.

Surprisingly, we found no effect of the exercise organs on Msum in the fall and summer. In fact, Msum variations in the fall appeared to relate to changes in digestive organ mass while in the summer no single organ was found to relate significantly to Msum. As for BMR, findings for the summer may be the result of a low sample size (n = 11) but that of the fall, despite the fact that digestive organs were found to be at their largest at that time, remains a mystery. These results should however be considered with caution since the number of individuals in the fall, for which we had Msum measures and data for all the organs, were similarly low (n = 12).

To conclude, our results are consistent with a predominant influence of the exercise organs on Msum variations (Cooper, 2002; Saarela & Hohtola, 2003; Swanson *et al.*, 2013; Vézina *et al.*, 2007) over the complete year and with the effect of skeletal muscles on Msum variability during winter. However, we found little support for an effect of the digestive organs on BMR variations, which contradicts earlier findings (Liu & Li, 2006; Williams & Tieleman, 2000), but rather a consistent influence of muscle mass and an effect of the excretory organs at peak of winter.

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CHAPITRE V

L'augmentation hivernale de la capacité thermogénique améliore la survie intra-hivernale des oiseaux de petite taille

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Increasing winter maximal metabolic rate improves intra-winter survival in small birds

Magali Petit*[1], Clavijo-Baquet Sabrina[2] and François Vézina[1]

[1]Département de Biologie, chimie et géographie, Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski (QC), G5L3A1, Canada. Groupe de recherche sur les environnements nordiques BORÉAS, Rimouski (QC), Canada. Centre d'Études Nordiques, Québec (QC), Canada. Centre de la Science de la Biodiversité du Québec, Montréal (QC) Canada. [2]Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile.

* Corresponding author. E-mail: magali.petit@uqar.ca

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Contributions - Conception de l'expérience: MP, FV ; Réalisation de l'expérience: MP, SCB; Analyse des données: MP, SCB ; Financement: FV ; Rédaction : MP, FV.

5.1 Résumé

En hiver, les espèces résidant sous les hautes latitudes augmentent leur métabolisme, ce qui est supposé améliorer leur survie. Cependant, le lien entre la performance métabolique hivernale et la survie reste à démontrer. Nous avons utilisé un protocole de capture-marquage-recapture pour suivre une population de mésanges à tête noire (*Poecile atricapillus*) en milieu naturel sur trois années afin d'évaluer leur survie intra- et inter-hivernale. Pour chaque oiseau, nous avons aussi mesuré la masse corrigée par la taille (M_s), l'hématocrite (Hct), le métabolisme de base (BMR) ainsi que la capacité thermogénique maximale (Msum) afin d'analyser l'effet de ces paramètres physiologiques sur la survie intra-hivernale et à long terme. Nos résultats ont montré que la probabilité de survie des mésanges était élevée et constante au sein de l'hiver (0,92) ainsi qu'à long terme (0,96). Nous avons aussi observé que tandis que M_s, Hct et BMR n'avaient pas d'effet sur la survie, la probabilité de survie intra-hivernale des mésanges était positivement reliée à leur Msum selon une courbe sigmoïde. Les individus exprimant un Msum inférieur à 1,26 W (*i.e.* similaire à la valeur estivale) avaient moins de 50% de chance de survie alors que les oiseaux avec un Msum supérieur à 1,35 W avaient plus de 90% de chance de survivre à l'hiver. Nos données suggèrent donc que les individus trop lents ou incapables d'ajuster leur phénotype entre l'été et l'hiver ont peu de chance de survie et donc que l'augmentation saisonnière de la performance métabolique est bénéfique aux mésanges. Cette étude montre, pour la première fois chez une population naturelle d'oiseaux, une relation positive entre le métabolisme hivernal et la survie, un estimé de la valeur sélective.

Mots-clefs: métabolisme ; BMR ; Msum ; survie; acclimatation hivernale; valeur sélective, mésange à tête noire

5.2 Abstract

Small resident bird species living at northern latitudes increase their metabolism in winter and this is widely assumed to improve their chances of survival. However, the relationship between winter metabolic performance and survival has yet to be demonstrated. Using capture-mark-recapture, we followed a population of free-living Black-capped chickadees (*Poecile atricapillus*) over three years and evaluated their survival probability within and among winters. We also measured the size-independent body mass (M_s), hæmatocrit (Hct), basal metabolic rate (BMR) and maximal thermogenic capacity (Msum) and investigated how these parameters influenced survival within and among winters. Results showed that survival probability was high and constant both within (0.92) and among (0.96) winters. They also showed that, while M_s, Hct and BMR had no significant influence, survival was positively related to Msum, following a sigmoid relationship, within but not among winter. Birds expressing a Msum below 1.26 W (*i.e.* similar to summer level) had less than 50% chance of survival while birds with a Msum above 1.35 W had at least 90% chance of surviving through the winter. Our data therefore suggest that individuals that are either too slow or unable to adjust their phenotype from summer to winter have little chances of survival and thus that seasonal upregulation of metabolic performance is highly beneficial. This study is the first to document in an avian system the relationship between thermogenic capacity and winter survival, a proxy of fitness.

Keywords: metabolic rate, BMR, Msum, survival, winter acclimatization, fitness, Black-capped chickadee

5.3 Introduction

For resident bird species living at northern latitudes, winters are characterized by low temperatures, reduced food availability, short foraging days and long nights of fasting (McNamara *et al.*, 1990; Swanson, 2010). To face these challenging conditions, small species use several physiological adjustments such as increases in lean body mass (Petit *et al.*, 2014; Vézina *et al.*, 2006; Williams & Tieleman, 2000) and fat reserves (Cooper, 2002; Petit *et al.*, 2014; Swanson, 1991a), elevated basal (BMR) and summit metabolic rates (Msum) (Cooper & Swanson, 1994; Petit *et al.*, 2013) as well as increased hæmatocrit (Hct) (Petit & Vézina, 2014a; Swanson, 1990b).

Total body mass (M_b), muscle mass and fat reserves are proxies of individual condition (Norte *et al.*, 2009). BMR, interpreted here as a measure of physiological maintenance costs, is assumed to vary with changes in size of digestive organs and muscles (Chappell *et al.*, 1999; Liknes & Swanson, 2011b; Petit *et al.*, 2014; Williams & Tieleman, 2000; Zheng *et al.*, 2008) while Msum, a measure of maximal thermogenic capacity, is mainly related to variations in pectoral muscle size and function (Liknes & Swanson, 2011a; Petit & Vézina, 2014a; Swanson *et al.*, 2013). Hct, the proportion of red blood cells in total blood, is a measure of maximal oxygen carrying capacity. It is related to overall metabolic activity (Carpenter, 1975; Swanson, 1990b) and thus varies positively with BMR and Msum (Burness *et al.*, 1998; Petit & Vézina, 2014a). Consequently, birds with higher lean mass, larger fat reserves and higher BMR, Msum and Hct are assumed to be best equipped to perform in a cold environment and to have better survival relatively to birds with lower physiological condition (Brodin, 2007; Pravosudov & Lucas, 2001; Verhulst *et al.*, 2004). However, although a positive relationship between these parameters and winter survival is commonly assumed (Nzama *et al.*, 2010; Petit *et al.*, 2013; Sears *et al.*, 2006; Swanson & Olmstead, 1999) and has been observed in some small mammals (Boratyński *et al.*, 2010; Jackson *et al.*, 2001), it has yet to be demonstrated in free-living birds (Swanson & Vézina, 2015).

In this study, we applied a capture-marking-recapture method to track 231 free-living Black-capped chickadees (*Poecile atricapillus*) wintering in eastern Canada from the winter of 2009-10 to the winter of 2011-12. We used measurements of M_b, body size, BMR, Msum and Hct together with recapture and resighting data to investigate, for the first time in birds,

the relationship between winter physiological condition and survival both within winter and among years.

Assuming that wintering birds increase their lean mass, fat reserves, metabolic performance and Hct to improve their survival and that high condition reflects high individual quality, we expected positive relationships between physiological parameters and survival within winter as well as among years. However, according to the resource allocation principle (Weiner, 1992), birds facing challenging conditions, such as winter, could show physiological tradeoffs to support their main activities (*i.e.* thermogenesis and foraging) at the expense of other physiological systems (*e.g.* immunity, self-maintenance) (Ardia & Clotfelter, 2007; Buehler *et al.*, 2009; Wiersma *et al.*, 2004). Hence, the energy invested in developing and maintaining the winter phenotype could also potentially compromise winter survival (*e.g.* by a reduction of self maintenance) and result in long term negative carry-over effects on fitness (Fretwell, 1972; Saino *et al.*, 2004). Consequently, we assumed the relationship between winter phenotype and survival to be non-linear, with maximal survival reached at an intermediate level of winter performance.

5.4 Materials and methods

5.4.1 Capture and handling

Our model species, the Black-capped chickadee, is a small (9-14g), non-migratory, territorial, philopatric and short lived (3 years) passerine (Schubert *et al.*, 2007; Schubert *et al.*, 2008; Smith, 1991; Weise & Meyer, 1979), which makes it perfectly suited for this study. The study was carried out in the Forêt d'Enseignement et de Recherche Macpès, Québec, Canada (48°18' N, 68°31' W) from October 2009 to December 2011. To facilitate capture and resighting, six feeding stations were set up during the first year and five stations were added during the second year, with an average distance between stations of 1.9 km. Each station included three metal feeders (Perky-Pet® 10" Sunflower Seed and Peanut Feeder) filled with black sunflower seeds. During captures (between 08:00 and 13:00), feeders were removed and homemade potter traps (15 cm x 15 cm x 15 cm) baited with seeds were used to catch birds. During the first winter (Sep 2009 to Mar 2010), we caught 139 birds (cohort 1), of which 72 were

relocated in the second year (Sep 2010 to Mar 2011) and 46 during the third year (Nov 2011 to Dec 2011). During the second year, we also caught 92 new birds (cohort 2) and 33 were resighted in the third year. Birds caught for the first time were banded with a USGS numbered metal band and a unique combination of three colored bands for further identification from a distance. At each capture, we collected a blood sample within less than 10 min by puncturing the brachial vein with a 26-gauge needle. Samples (total blood volume <120 µl) were kept cold (< 4°C but above freezing) in heparanized microcapillary tubes until centrifugation (10 min at 16,000 g) and Hct measurements. After blood sampling, birds were weighed and were measured following standardized protocols (length of head plus beak, tarsus and wing measured) (Mandin & Vézina, 2012; Petit *et al.*, 2013). We used the shape and wear of the sixth rectrices to determine the age (young or adult) of birds (Pyle *et al.*, 1987). Following these measurements, up to four birds per day were brought to the field station for metabolic trials. All bird manipulations were approved by the animal care committee of the Université du Québec à Rimouski (CPA-37-09-68) and have been conducted under scientific and banding permits from Environment Canada - Canadian Wildlife Service.

5.4.2 Respirometry

At the field station, birds were kept in separate cages (39 cm x 43 cm x 31 cm) in a quiet room receiving natural light, with an *ad libitum* access to food (sunflower seed) and water until metabolic rate measurements. At around 13:00, we began to measure the Msum of two birds in parallel using the instruments and protocol described by Petit *et al.* (2013). Measurements of the two remaining birds started before 15:00. For each sequence we weighed (± 0.1 g) the birds and recorded their body temperature (T_b) with a thermocouple reader (Omega model HH-25KC, NIST-traceable, Omega, Montréal, QC, Canada) using a copper-constantan thermocouple inserted into the cloacae approximately 10 mm deep. Then, birds were put in metabolic chambers (effective volume = 1120 ml) fitted with a perch and a thermistor (Sable Systems UI2 AD converter, Sable Systems, Las Vegas, NV, USA) for chamber temperature measurements. We exposed the birds to helox gas (21% oxygen, 79% helium, average flow rate of 1109 $ml.min^{-1}$) and measured their oxygen consumption (FoxBox oxygen analyzers, Sable Systems, Las Vegas, NV, USA) using a sliding cold

exposure protocol (Swanson *et al.*, 1996). This procedure consisted in decreasing ambient temperature by 3°C every 20 minutes, with trials starting at 0°C. Trials ended when birds became hypothermic, which was detectable in real time as a steady decline in oxygen consumption for several minutes, time at which we measured the bird's M_b and T_b again. We assumed a bird had reached its Msum when T_b after a trial was ≤ 38°C (Cooper & Gessaman, 2005) (mean T_b after Msum = 33.4 ± 0.3°C). Data from birds with T_b above this threshold were discarded. M_b measured before and after trials were averaged and these values were used for Msum analyses. Birds were brought back to their cage with food and water *ad libitum* until BMR measurement.

We measured the BMR of the four birds simultaneously from 19:00 to 06:00. Measurements were done at 30°C (within the thermoneutral zone for this species, Chaplin, 1976) using a constant flow of CO_2-free dry air (average 470 $ml.min^{-1}$). As for Msum, birds were weighed before and after measurements and average M_b were used in BMR analyses.

Oxygen analyzers were adjusted each day to 20.95% O_2 using CO_2-free dry air. Mass flow valves (Sierra Instruments, Side-Trak® Model 840, Monterey, CA, USA) were calibrated for air and helox using a bubble-O-meter (Dublin, OH, USA) once *per* winter. Metabolic rate calculations were done with ExpeData software, v1.2.6 (Sable Systems, Las Vegas, NV, USA). Using a 20 sec sampling interval for BMR and a 5 sec interval for Msum, BMR and Msum calculations were based respectively on the lowest and highest averaged 10 minutes of oxygen consumption *per* measurement sequence according to Lighton's equation 10.1 (Lighton, 2008). We applied the instantaneous measurement technique (Bartholomew *et al.*, 1981) for Msum calculations and a steady state approach for BMR. Duration of BMR trials (around 11 hours) insured that birds were post-absorptive at time of BMR measurement. Since birds use lipids as fuel during fasting and for shivering (Swanson, 2010), we estimated energy consumption using a constant equivalent of 19.8 $kJ.L^{-1}$ O_2 and converted to watts (Gessaman & Nagy, 1988). After BMR measurements, birds were put back in their cage with access to food and water until release on their capture site around 2 hours later. In total, 180 Msum and 221 BMR were recorded for 101 and 114 individuals, respectively.

5.4.3 Capture-Mark-Recapture (CMR) method

Each bird was marked at capture and released in the population after metabolic measurements. Besides capture sessions, to maximize our probability of resighting marked individuals, we also carried out an average of 1.9 ± 0.1 observation sessions of one hour *per* month on each site over the whole period of the study. We used both capture and observation from a distance as encounter occasions to calculate the return rate of birds, which depends on their probability of 1) surviving and coming back to the sampling site (Φ, the apparent survival probability) and 2) being encountered (p, the encounter probability). Using this protocol, birds from both cohorts were caught and resighted during the winter of their first capture (encounter occasions per bird within the first winter: cohort 1 = 2.5 ± 0.1; cohort 2 = 2.0 ± 0.2) and, for cohort 1, also during the following two winters (total encounter occasions per bird of cohort 1 = 4.1 ± 0.2). CMR protocol allowed us to record the encounter history of each bird. For example, an encounter history of "101" meant that a bird had been caught at the first encounter occasion, missed during the second occasion and resighted during the third occasion. To calculate short-term survival (*i.e.* within winter survival), we used CMR data collected for both cohorts during the winter of their first capture (*i.e.* winter 2009/2010 for cohort 1 and winter 2010/2011 for cohort 2) while we calculated long-term survival (*i.e.* among year survival) using CMR data of the cohort 1 collected from Sep 2009 to Dec 2011.

5.4.4 Statistical analysis

5.4.4.1 Data preparation

Total body mass and size-independent body mass (M_s) are expected to be related to individual condition (Norte *et al.*, 2009). However, because they were highly correlated ($r = 0.80$), we could not include both variables in the analyses and therefore used only M_s as a measure of condition. Besides a seasonal increase in lean mass and fat reserves (Petit *et al.*, 2013, 2014), wintering Black-capped chickadees also follow a daily fattening cycle during which they store fat from sunrise to sunset, which is depleted during the night (Lehikoinen, 1987; Mandin & Vézina, 2012). Consequently, M_s in our birds depended on the time they were caught both during the winter and during the day. Hence, to calculate M_s, we first performed a principal component analysis on morphological data (length of head plus beak,

wing and tarsus; Rising & Somers, 1989) to obtain an index (PC1) of structural body size (hereafter named "size"). Then, we added size ($F_{1,595} = 333.7$; $p < 0.0001$) in a GLM testing for the effects of date ($F_{1,595} = 14.5$; $p < 0.001$) and the variable "relative time of capture" (time since sunrise / day length, hereafter "time of capture") ($F_{1,595} = 79.5$; $p < 0.0001$) on M_b and used the residuals as our values of M_s.

BMR was dependent on M_b ($F_{1, 222} = 46.0$; $p < 0.0001$) but not on date ($p = 0.3$) while Msum was influenced by both M_b ($F_{1, 178} = 31.7$; $p < 0.0001$) and date ($F_{1, 178} = 13.9$; $p < 0.001$). We therefore conducted two sets of survival analyses, one including raw BMR and Msum and the other including residual BMR and residual Msum, controlling for the effect of M_b and date.

We used raw Hct values in survival analyses since Hct was not related to date ($p = 0.7$), time of capture ($p = 0.5$), M_b ($p = 0.3$) or size ($p = 0.6$).

When a bird was measured several times within a winter (for within winter analyses) or in more than one winter (for the among year analyses), we calculated the mean of each physiological parameter for a given bird and used these means in the survival analyses.

Our analyses were therefore conducted using both raw and residuals of BMR and Msum. However, when one of the residual parameters was found to significantly affect apparent survival, we reported corresponding uncorrected values in the text and figures to allow for comparison with other studies. These analysis were performed with R version 3.0.3 (R Core Team, 2014).

5.4.4.2 Selection of the basic model

M_s, Hct, BMR and Msum were available for 81 individuals for the within winter survival analysis and for 56 individuals for the among year analysis, we therefore restricted our databases to these birds for further analyses.

Survival analyses were performed using the software MARK version 7.2 (White & Burnham, 1999). To analyse within winter survival ($n = 81$), we used data recorded during the first winter for cohort 1 and data from the second winter for cohort 2. For both winters, we grouped encounter occasions in 3 periods (period 1 = Sept to Nov, period 2 = Dec to Jan;

period 3 = Feb to Mar; see table A.7 in Annexes). Cormack-Jolly-Seber (CJS) models are based on live captures and releases of marked individuals into the population. We therefore analysed the effects of cohort (1 and 2), age at first capture (juvenile and adult) and period on the return rate by testing for an effect of group (group 1 = cohort 1/Adult, group 2 = cohort1/Juvenile; group 3 = cohort 2/Adult; group 4 = cohort 2/ Juvenile) and period on both survival and encounter probabilities (return rate = $\Phi_{(gxt)}p_{(gxt)}$).

For among year survival analyses (n = 56), we only used data from cohort 1. We grouped encounter data in seven periods (period 1 = Sept to Nov 2009, period 2 = Dec 2009 to Jan 2010; period 3 = Feb to Mar 2010, period 4 = Sept to Nov 2010, period 5 = Dec 2010 to Jan 2011; period 6 = Feb to Mar 2011, period 7 = Nov to Dec 2011; see table A.8 in Annexes). We analyzed the relationship between age at first capture, periods and return rate by testing the effect of group (group 1 = Adult; group 2 = Juvenile) and period on both survival and encounter probabilities (return rate = $\Phi_{(gxt)}p_{(gxt)}$).

We first tested whether our data fitted the full time-dependent CJS model (return rate = $\Phi_{(gxt)}p_{(gxt)}$) using the median ĉ estimator provided by MARK to estimate the overdispersion of our data. As the median overdispersion factor (ĉ) was always inferior to 3, we used CJS models for further analyses. However, when median ĉ was superior to 1, we multiplied the variance-covariance matrix by median ĉ to control for the overdispersion of our data.

For both within winter and among year survival analyses, the model with the lowest 2nd order Aikake information criteria (AICc or QAICc when the matrix was multiplied by median ĉ) was selected as the basic model. In the within winter analyses, the effects of group and period were not significant (see Results 5.5), we therefore used the null model (return rate = $\Phi_{(\cdot)}p_{(\cdot)}$) as basic model for these analyses. In the among year analyses, the effect of period was significant on the encounter probability (see Results 5.5), we therefore used the model return rate = $\Phi_{(\cdot)}p_{(t)}$ as basic model for the among year analyses.

5.4.4.3 Effect of physiological parameters on survival

We analysed the effect of physiological condition on both within winter and among year survival by including average M_s, Hct, BMR and Msum (raw and residuals considered in separate sets of analyses) as covariates in the basic models (full model within winter: $\Phi_{(Ms+Ms^2+Hct+Hct^2+BMR+BMR^2+Msum+Msum^2)}p_{(\cdot)}$, among years: $\Phi_{(Ms+Ms^2+Hct+Hct^2+BMR+BMR^2+Msum+Msum^2)}p_{(t)}$) and we selected the model with the lowest AICc (or QAICc) as the best model. Then, we used likelihood ratio tests to determine the significance of the covariates. The software MARK outputs survival probabilities for predicted values. This means that, when using residual BMR and residual Msum in our analyses, MARK provided survival probabilities for newly generated residuals of BMR and Msum. These new residuals are not statistically controlling for additional variables and remain in the same units as the original variables included in the model. To report equivalent uncorrected BMR and Msum, we therefore used our complete databases (BMR: n = 221, Msum: n = 180) and ran regressions between raw values and their residuals (controlling for M_b and date, see Results 5.5). Then, we used the regression equations to recalculate uncorrected values from residuals produced by MARK.

5.5 Results

5.5.1 Within winter survival

Within winter return rate did not depend on group ($X^2 = 5.1$, $p = 0.2$) or period ($X^2 = 0.5$, $p = 0.5$). Based on the AICc, the best model explaining our data was the null model $\Phi_{(\cdot)}p_{(\cdot)}$ (table 5.1). Hence, for both cohorts combined together, the apparent survival probability as well as the encounter probability were high and constant throughout winter and age ($\Phi = 0.92 \pm 0.05$; $p = 0.86 \pm 0.06$, figure 5.1).

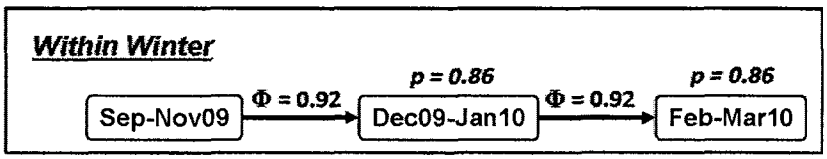

Figure 5.1 Diagram representing both the apparent survival (Φ) and the encounter probabilities (p) within winter. Apparent survival and encounter probability are constant.

Table 5.1 Model selection testing for the effect of period and group (*i.e.* cohort and age) on the return rate of both cohorts during winter. (W_{AICc} : weight of the model, Par : number of parameters, Dev.: Deviance)

N°	Model	AICc	ΔAICc	W_{AICc}	Likelihood	Par	Dev.
1	**Φ(.) p(.)**	**87.1**	**0.0**	**0.3**	**1.00**	**2**	**14.9**
2	Φ(g) p(.)	88.7	1.6	0.1	0.45	5	9.8
3	Φ(.) p(t)	88.8	1.7	0.1	0.43	3	14.4
4	Φ(t) p(.)	88.8	1.7	0.1	0.43	3	14.4
5	Φ(t) p(t)	88.8	1.7	0.1	0.43	3	14.4
6	Φ (g) p(g)	89.9	2.8	0.1	0.24	7	6.2
7	Φ (g) p(t)	90.8	3.7	0.0	0.16	6	9.5
8	Φ (.) p(g)	92.0	4.9	0.0	0.09	5	13.1
9	Φ (t) p(g)	93.5	6.4	0.0	0.04	6	12.3
10	Φ (g*t) p(t)	94.9	7.8	0.0	0.02	8	8.6
11	Φ (t) p(g*t)	95.1	8.0	0.0	0.02	8	8.8
12	Φ (.) p(g*t)	95.3	8.2	0.0	0.02	8	9.0
13	Φ (g*t) p(.)	95.3	8.2	0.0	0.02	8	9.1
14	Φ (g*t) p(g*t)	96.3	9.2	0.0	0.01	10	4.8
15	Φ (g) p(g*t)	96.5	9.4	0.0	0.01	10	5.0
16	Φ (g*t) p(g)	97.6	10.5	0.0	0.01	10	6.1

mean $\hat{c}$ = 1.0

t, period with 3 levels: 1 = Sept to Nov, 2 = Dec to Jan; 3 = Feb to mar

g, group with 4 levels: 1 = cohort1/Adult ; 2 = cohort 1/Juvenile; 3 = cohort 2/Adult; 4 = cohort 2/Juvenile

Once we added covariates to the null model, we observed that the within winter return rate was best explained by a model where the survival probability was dependent on Hct and residuals of Msum ($\Phi_{(Hct+resMsum)}p_{(\bullet)}$, table 5.2). Analyses of the covariate effects showed that winter survival varied with residual Msum ($\Phi_{(\bullet)}p_{(\bullet)}$ *vs.* $\Phi_{(resMsum)}p_{(\bullet)}$: $X^2 = 10.7$, $p < 0.01$) and that the addition of Hct in the model improved survival probability ($\Phi_{(Hct+resMsum)}p_{(\bullet)}$ *vs.* $\Phi_{(resMsum)}p_{(\bullet)}$: $X^2 = 5.3$, $p < 0.05$) although Hct alone did not affect apparent survival ($\Phi_{(\bullet)}p_{(\bullet)}$ *vs.* $\Phi_{(Hct)}p_{(\bullet)}$: $X^2 = 1.7$, $p = 0.2$). Within winter survival was not related to M_s ($\Phi_{(\bullet)}p_{(\bullet)}$ *vs.* $\Phi_{(Ms)}p_{(\bullet)}$: $X^2 = 0.03$, $p = 0.96$) or residual BMR ($\Phi_{(\bullet)}p_{(\bullet)}$ *vs.* $\Phi_{(resBMR)}p_{(\bullet)}$: $X^2 = 0.1$, $p = 0.8$).

Table 5.2 Model selection testing for the effect of mean size-independent body mass (M_s), haematocrit (Hct), residual basal metabolic rate (resBMR) and residual summit metabolic rate (resMsum) on the return rate of both cohorts during winter. (W_{AICc}: weight of the model, Par : number of parameters, Dev.: Deviance)

N°	Model	AICc	ΔAICc	W_{AICc}	Likelihood	Par	Dev.
1	**Φ(Hct+resMsum) p(.)**	**76.0**	**0.0**	**0.3**	**1.00**	**4**	**67.4**
2	Φ(Hct+resBMR+resMsum) p(.)	76.6	0.6	0.2	0.73	5	65.7
3	Φ(M_s+M_s^2+Hct+Hct^2+resBMR+$resBMR^2$+resMsum+$resMsum^2$) p(.)	78.2	2.2	0.1	0.33	10	54.7
4	Φ(M_s+Hct+resMsum) p(.)	78.2	2.3	0.1	0.32	5	67.4
5	Φ(M_s+Hct+resBMR+resMsum) p(.)	78.7	2.7	0.1	0.26	6	65.4
6	Φ(resMsum) p(.)	79.1	3.1	0.1	0.21	3	72.7
7	Φ(resBMR+resMsum) p(.)	80.3	4.4	0.0	0.11	4	71.8
8	Φ(M_s+M_s^2) p(.)	80.7	4.7	0.0	0.09	4	72.1
9	Φ(M_s+resMsum) p(.)	80.7	4.8	0.0	0.09	4	72.2
10	Φ(M_s+resBMR+resMsum) p(.)	81.0	5.1	0.0	0.08	5	70.2
11	Φ(resMsum+$resMsum^2$) p(.)	81.1	5.2	0.0	0.08	4	72.6
12	Φ(Hct+Hct^2) p(.)	87.5	11.6	0.0	0.00	4	78.9
13	Φ(.) p(.)	87.5	11.6	0.0	0.00	2	83.4
14	Φ(Hct) p(.)	88.0	12.0	0.0	0.00	3	81.6
15	Φ(resBMR) p(.)	89.7	13.7	0.0	0.00	3	83.3
16	Φ(M_s) p(.)	89.7	13.8	0.0	0.00	3	83.4
17	Φ(M_s+Hct) p(.)	90.0	14.0	0.0	0.00	4	81.4
18	Φ(Hct+resBMR) p(.)	90.2	14.2	0.0	0.00	4	81.6
19	Φ(M_s+resBMR) p(.)	91.9	15.9	0.0	0.00	4	83.3
20	Φ(resBMR+$resBMR^2$) p(.)	91.9	15.9	0.0	0.00	4	83.3
21	Φ(M_s+Hct+resBMR) p(.)	92.2	16.2	0.0	0.00	5	81.3

mean $\hat{c}$ = 1.0

The relationship between Msum and residual Msum was linear and strong ($r = 0.89$, $n = 180$ $p < 0.0001$), allowing us to use the regression equation (Msum = 1.535+residual Msum) to back-calculate uncorrected Msum. The relationship between winter survival and uncorrected Msum followed a non-linear curve (figure 5.2a). Below an Msum of 1.17 W, winter survival probability was less than 10%, between 1.20 W and 1.32 W, survival probability increased linearly reaching 50% at 1.26 W. When birds expressed an average Msum above 1.35 W, winter survival probability was greater than 90% and individuals with an Msum superior to 1.46 W were expected to have 100% chances of survival.

Analyses with raw BMR and raw Msum showed that the within winter return rate was best explained by a model where the survival probability was dependent on BMR and Msum

($\Phi_{(BMR+Msum)}p_{(\bullet)}$, table 5.3). Analyses of the covariate effects revealed that within winter survival varied with Msum ($\Phi_{(\bullet)}p_{(\bullet)}$ *vs.* $\Phi_{(Msum)}p_{(\bullet)}$: $X^2 = 13.2$, $p < 0.001$) and that the addition of BMR in the model improved survival probability ($\Phi_{(BMR+Msum)}p_{(\bullet)}$ *vs.* $\Phi_{(Msum)}p_{(\bullet)}$: $X^2 = 15.1$, $p < 0.001$) although BMR alone did not affect apparent survival ($\Phi_{(\bullet)}p_{(\bullet)}$ *vs.* $\Phi_{(BMR)}p_{(\bullet)}$: $X^2 = 0.3$, $p = 0.6$). Within winter survival was not related to M_s ($\Phi_{(\bullet)}p_{(\bullet)}$ *vs.* $\Phi_{(Ms)}p_{(\bullet)}$: $X^2 = 0.03$, $p = 0.96$) or Hct ($\Phi_{(\bullet)}p_{(\bullet)}$ *vs.* $\Phi_{(Hct)}p_{(\bullet)}$: $X^2 = 1.7$, $p = 0.2$) in this case. The relationship between winter survival and raw Msum followed a sigmoid curve (figure 5.2b). Below an Msum of 1.35 W, within winter survival probability was null while above 1.35 W, within winter survival probability was 100%.

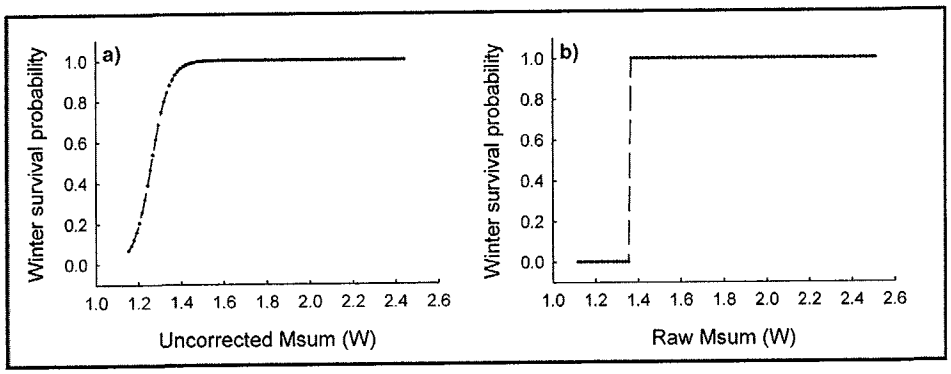

Figure 5.2 Relationship between winter apparent survival and a) winter uncorrected Msum, with survival controlled for the effect of hæmatocrit and b) winter raw Msum, with survival controlled for the effect of BMR (see text for details).

Table 5.3 Model selection testing for the effect of mean size-independent body mass (M_s), haematocrit (Hct), basal metabolic rate (BMR) and summit metabolic rate (Msum) on the return rate of both cohorts during winter. (W_{AICc}: weight of the model, Par : number of parameters, Dev.: Deviance)

N°	Model	AICc	ΔAICc	W_{AICc}	Likelihood	Par	Dev.
1	**Φ(BMR+Msum) p(.)**	**63.6**	**0.0**	**0.49**	**1.00**	**4**	**55.1**
2	Φ(Hct+BMR+Msum) p(.)	65.9	2.3	0.16	0.32	5	55.1
3	Φ(M_s+BMR+Msum) p(.)	65.9	2.3	0.16	0.32	5	55.1
4	Φ(M_s+Hct+BMR+Msum) p(.)	65.9	2.3	0.16	0.32	5	55.1
5	Φ(M_s+M_s^2+Hct+Hct^2+BMR+BMR^2+Msum+$Msum^2$) p(.)	68.9	5.2	0.04	0.07	7	53.2
6	Φ(Hct+Msum) p(.)	76.2	12.6	0.00	0.00	4	67.6
7	Φ(Msum) p(.)	76.5	12.9	0.00	0.00	3	70.2
8	Φ(M_s+Hct+Msum) p(.)	78.5	14.9	0.00	0.00	5	67.6
9	Φ(M_s+Msum) p(.)	78.7	15.1	0.00	0.00	4	70.2
10	Φ(Msum+$Msum^2$) p(.)	78.7	15.1	0.00	0.00	4	70.2
11	Φ(M_s+M_s^2) p(.)	80.7	17.1	0.00	0.00	4	72.1
12	Φ(Hct+Hct^2) p(.)	87.5	23.9	0.00	0.00	4	79.0
13	Φ(.) p(.)	87.5	23.9	0.00	0.00	2	83.4
14	Φ(Hct) p(.)	88.0	24.3	0.00	0.00	3	81.6
15	Φ(BMR) p(.)	89.4	25.8	0.00	0.00	3	83.1
16	Φ(M_s) p(.)	89.7	26.1	0.00	0.00	3	83.4
17	Φ(M_s+Hct) p(.)	90.0	26.3	0.00	0.00	4	81.4
18	Φ(Hct+BMR) p(.)	90.1	26.5	0.00	0.00	4	81.6
19	Φ(BMR+BMR^2) p(.)	91.4	27.8	0.00	0.00	4	82.8
20	Φ(M_s+BMR) p(.)	91.6	28.0	0.00	0.00	4	83.1
21	Φ(M_s+Hct+BMR) p(.)	92.2	28.6	0.00	0.00	5	81.3

mean $\hat{c}$ = 1.0

5.5.2 Among year survival

Among years, the return rate did not depend on group (X^2 = 0.9, p = 0.3) but was influenced by the period (X^2 = 12.8, $p < 0.05$). Based on the QAICc, the best model explaining our data was the model $\Phi_{(\cdot)}p_{(t)}$ (table 5.4). Hence, the apparent survival probability was high and constant among periods and ages (Φ = 0.96 ± 0.02) but the encounter probability varied over time. Specifically, encounter probability decreased between the end of a winter and the beginning of the next one (figure 5.3).

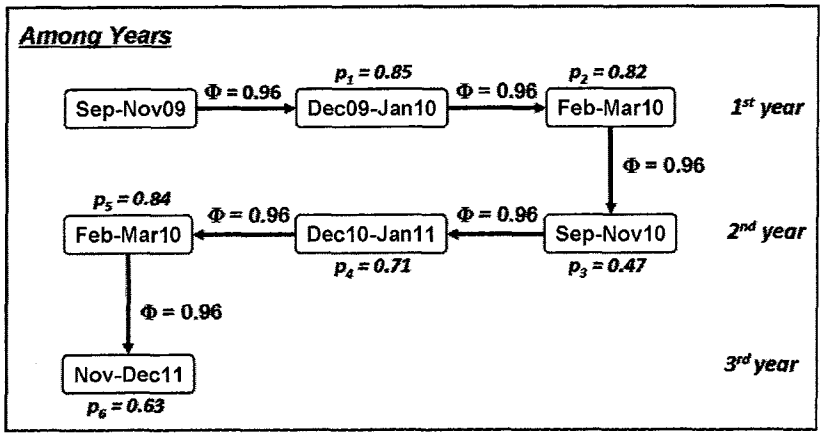

Figure 5.3 Diagram representing both the apparent survival (Φ) and the encounter probabilities (p) among years. Apparent survival was constant while encounter probabilities decreased between the end of a winter and the beginning of the next one.

With the covariates included in the model $\Phi_{(\cdot)}p_{(t)}$, among year return rate was best explained by a model including residuals of Msum and size-independent body mass ($\Phi_{(Ms+resMsum)}p_{(t)}$, table 5.5). However, the effects of residual Msum and M_s alone or combined were not significant ($\Phi_{(\cdot)}p_{(t)}$ *vs.* $\Phi_{(resMsum)}p_{(t)}$: $X^2 = 2.7$, $p = 0.1$; $\Phi_{(\cdot)}p_{(t)}$ *vs.* $\Phi_{(Ms)}p_{(t)}$: $X^2 = 2.8$, $p = 0.1$; $\Phi_{(\cdot)}p_{(t)}$ *vs.* $\Phi_{(Ms+resMsum)}p_{(t)}$: $X^2 = 5.8$, $p = 0.06$). Among year return rate was not dependent on Hct ($\Phi_{(\cdot)}p_{(t)}$ *vs.* $\Phi_{(Hct)}p_{(t)}$: $X^2 = 0.0$, $p = 1.0$) or on residuals of BMR ($\Phi_{(\cdot)}p_{(t)}$ *vs.* $\Phi_{(BMR)}p_{(t)}$: $X^2 = 0.2$, $p = 0.6$). Analyses with raw BMR and raw Msum gave the same results (not shown). Therefore, long-term survival was not dependent on any of the measured physiological parameter.

Table 5.4 Model selection testing for the effect of event and age on the return rate of cohort 1 between years. (QAICc: modified AICc, W_{QAICc}: weight of the model, Par : number of parameters, QDev.: deviance)

N°	Model	QAICc	ΔQAICc	W_{QAICc}	Likelihood	Par	QDev.
1	**Φ(.) p(t)**	**223.2**	**0.0**	**0.4**	**1.00**	**7**	**77.4**
2	Φ(g) p(t)	224.7	1.4	0.2	0.49	8	76.7
3	Φ(.) p(.)	225.5	2.2	0.1	0.33	2	90.2
4	Φ(t) p(t)	225.7	2.5	0.1	0.29	11	71.0
5	Φ(g) p(.)	226.6	3.4	0.1	0.19	3	89.3
6	Φ(.) p(g)	227.4	4.2	0.1	0.12	3	90.1
7	Φ(g) p(g)	228.7	5.5	0.0	0.07	4	89.3
8	Φ(t) p(.)	231.3	8.1	0.0	0.02	7	85.5
9	Φ(t) p(g)	233.4	10.1	0.0	0.01	8	85.4
10	Φ(.) p(g*t)	233.8	10.6	0.0	0.01	13	74.5
11	Φ(g) p(g*t)	235.9	12.7	0.0	0.00	14	74.3
12	Φ(t) p(g*t)	236.0	12.7	0.0	0.00	17	67.2
13	Φ(g*t) p(t)	237.7	14.5	0.0	0.00	17	68.9
14	Φ(g*t) p(.)	242.0	18.8	0.0	0.00	13	82.7
15	Φ(g*t) p(g)	244.3	21.1	0.0	0.00	14	82.7
16	Φ(g*t) p(g*t)	247.3	24.1	0.0	0.00	22	66.0

mean $\hat{c}$ = 1.557

t, period with 7 levels: 1 = Sept to Nov 2009, 2 = Dec 2009 to Jan 2010; 3 = Feb to Mar 2010, 4 = Sept to Nov 2010, 5 = Dec 2010 to Jan 2011; 6 = Feb to Mar 2011, 7 = Nov to Dec 2011.

g, group with 2 levels : 1 = Adult ; 2 = Juvenile

Table 5.5 Model selection testing for the effect of mean size-independent body mass (M_s), haematocrit (Hct), residual basal metabolic rate (resBMR) and residual summit metabolic rate (resMsum) on the return rate of cohort 1 among years. (QAICc: modified AICc, W_{QAICc}: weight of the model, Par : number of parameters, QDev.: Deviance).

N°	Model	QAICc	ΔQAICc	W_{QAICc}	Likelihood	Par	QDev.
1	**Φ(M_s+resMsum) p(t)**	**227.0**	**0.0**	**0.2**	**1.00**	**9**	**208.0**
2	Φ(M_s) p(t)	227.8	0.8	0.1	0.68	8	211.0
3	Φ(resMsum) p(t)	227.9	0.9	0.1	0.63	8	211.1
4	**Φ(.) p(t)**	**228.4**	**1.4**	**0.1**	**0.49**	**7**	**213.8**
5	Φ(M_s+resBMR+resMsum) p(t)	229.2	2.2	0.1	0.33	10	208.0
6	**Φ(M_s+Hct+resMsum) p(t)**	**229.2**	**2.2**	**0.1**	**0.33**	**10**	**208.0**
7	Φ(M_s+resBMR) p(t)	229.6	2.6	0.1	0.27	9	210.6
8	**Φ(M_s+M_s^2) p(t)**	**229.9**	**2.9**	**0.0**	**0.24**	**9**	**210.9**
9	Φ(resMsum+resMsum^2) p(t)	229.9	2.9	0.0	0.23	9	210.9
10	**Φ(M_s+Hct) p(t)**	**230.0**	**3.0**	**0.0**	**0.23**	**9**	**211.0**
11	Φ(Hct+resMsum) p(t)	230.0	3.0	0.0	0.22	9	211.0
12	**Φ(resBMR+resMsum) p(t)**	**230.1**	**3.1**	**0.0**	**0.21**	**9**	**211.1**
13	Φ(resBMR) p(t)	230.4	3.4	0.0	0.18	8	213.6
14	**Φ(Hct) p(t)**	**230.6**	**3.6**	**0.0**	**0.16**	**8**	**213.8**
15	Φ(M_s+Hct+resBMR+resMsum) p(t)	231.5	4.5	0.0	0.11	11	208.0
16	**Φ(M_s+Hct+resBMR) p(t)**	**231.8**	**4.8**	**0.0**	**0.09**	**10**	**210.6**
17	Φ(.) p(.)	231.9	4.9	0.0	0.09	2	227.8
18	**Φ(Hct+resBMR+resMsum) p(t)**	**232.2**	**5.3**	**0.0**	**0.07**	**10**	**211.0**
19	Φ(resBMR+resBMR^2) p(t)	232.4	5.4	0.0	0.07	9	213.4
20	**Φ(Hct+Hct^2) p(t)**	**232.5**	**5.5**	**0.0**	**0.06**	**9**	**213.5**
21	Φ(Hct+resBMR) p(t)	232.6	5.6	0.0	0.06	9	213.6
22	Φ(M_s+M_s^2+Hct+Hct^2+resBMR+resBMR^2+resMsum+ resMsum^2) p(t)	239.6	12.6	0.0	0.00	15	206.9

mean $\hat{c}$ = 1.557

t, period with 7 levels: 1 = Sept to Nov 2009, 2 = Dec 2009 to Jan 2010; 3 = Feb to Mar 2010, 4 = Sept to Nov 2010, 5 = Dec 2010 to Jan 2011; 6 = Feb to Mar 2011, 7 = Nov to Dec 2011

5.6 Discussion

In this study, we investigated the effect of winter metabolism on survival of free-living Black-capped chickadees. We expected a positive and non-linear effect of winter physiological condition on both within winter and among year survivals. Our results showed that within winter survival was indeed positively and non-linearly related to maximal thermogenic capacity. However, survival among years was not dependent on any of the measured physiological parameter. This study is therefore the first to show the benefit of increasing winter thermogenic capacity for survival, a proxy of fitness, in free-living birds.

High winter survival in Black-capped chickadees

Apparent survival probability was high (0.92) within winter and remained constant throughout the season despite the seasonal peak of cold occurring in midwinter (see Petit *et al.*, 2013). This finding is consistent with observations in chickadees made by others (Desrochers *et al.*, 1988; Egan & Brittingham, 1994), including in food-supplemented populations (Egan & Brittingham, 1994). Long term apparent survival was also high (0.96) and constant among years. Loery *et al.* (1997) and Schubert *et al.* (2008) suggested that differences in survival between years are resulting from environmental variability, such as change in food availability or in habitat. Given the lack of year effect on survival, our results therefore imply that our population of Black-capped chickadees faced a relatively stable environment during the three years of the study.

Whether we considered it within or among winters, survival was not related to age. Since the highest rates of mortality in juvenile birds occurs during the nestling and fledgling stages (Perrins, 1965; Smith, 1967; Sullivan, 1989), this finding suggests that the juvenile chickadees that were captured during winter had presumably passed this critical point and were equally likely to survive than the adults.

Influence of metabolic performance on survival

Several studies made the assumption that by increasing metabolic performance, wintering birds improve their chances of survival (Nzama *et al.*, 2010; Petit *et al.*, 2013; Sears *et al.*, 2006; Swanson & Olmstead, 1999). We therefore expected to find positive but non-linear relationships between survival and winter levels of both BMR and Msum in Black-capped chickadees. Furthermore, since it appears to support thermogenic capacity (Petit & Vézina, 2014a), we also expected a positive relationship between survival and Hct. Our results only partially supported these predictions. They showed that BMR and Hct alone did not affect survival probability but that birds expressing a high Msum were more likely to survive through a given winter (and even more so if they had a high Hct). Winter acclimatization is associated with the growth of several internal organs in small birds (Liknes & Swanson, 2011b; Liu & Li, 2006; Petit *et al.*, 2014; Zheng *et al.*, 2008). It is thought that this organ development supports a seasonal increase in foraging as well as general thermoregulation needs and that this in turn leads to an increase in basal metabolism (Cooper & Swanson, 1994; Petit *et al.*, 2013). As we found no influence of winter BMR alone on survival, it appears, however, that this enhanced support capacity and the associated benefit for heat production are not sufficient to improve chances of winter survival. Similarly, although mid range Hct levels seem optimal to support high winter Msum (around 0.53, parabolic relationship, see Petit & Vézina, 2014a), this parameter is nevertheless quite variable among chickadees expressing high Msums (Petit & Vézina, 2014a). Winter Hct may therefore have to reach extreme values (*e.g.* severe anaemia) before an effect on survival can be observed.

Birds improve their thermogenic capacity ahead of time to increase their chances of winter survival

Within winter survival was positively related to maximal thermogenic capacity. Birds measured during winter that had an average uncorrected Msum below 1.26 W, representing 5% of our population, had less than 50% chance of survival while birds with an average uncorrected Msum above 1.35 W, representing 95% of our population, had at least 90% chance of surviving through the winter (figure 5.2a). Using raw Msum rather than residual Msum in our analyses resulted in the same observation, although the relationship between

winter survival and maximal thermogenic capacity was less nuanced. In this case, the cutoff value for survival was 1.35 W. Below this point, birds had no chance of survival (15% of our population) and above this point birds were expected to survive the winter season (85% of the population, figure 5.2b). In previous studies on the same chickadee population (Petit *et al.*, 2013, 2014), we found that summer (August) levels of raw Msum were on average 1.26 W (unpublished data), a value that should confer to wintering individuals less than 50% chance of survival when considering the effect of body mass and date on Msum. We also showed that Msum begins to increase before October (Petit *et al.*, 2013) and perhaps even as early as the end of August (Petit & Vézina, 2014b) in this species. Therefore, not only these birds apparently have to increase their thermogenic capacity well ahead of the peak of winter cold but it also seems that individuals that are either too slow or unable to adjust their phenotype from summer to winter have less than 50% chance of surviving. As temperature is the main driver of Msum variations in this and other species (Petit & Vézina, 2014b; Swanson *et al.*, 2014b; Swanson & Olmstead, 1999), it is likely that thermogenic capacity begins to increase as soon as the birds encounter the first cold nights of the early autumn (Petit *et al.*, 2013). The actual level of heat loss required to trigger this upregulation remains, however, to be investigated. Our data therefore confirm for the first time in birds (Boratyński *et al.*, 2010; Jackson *et al.*, 2001) that increasing thermogenic capacity does improve winter survival in small-bodied species.

Why maintain a higher Msum than needed for survival?

We predicted that chickadees would express maximal survival probability at intermediate Msum levels. However, although the relationship between survival and thermogenic capacity was positive and non-linear, apparent survival was not compromised when Msum reached its highest level. Indeed, our data suggest that winter Msum could be maintained at average levels well above 1.35 W (90% survival) or even above 1.46 W, the threshold value associated with 100% survival (figure 5.2). Accordingly, in a separate group of birds measured in February 2011, average level of Msum was 1.58 W (based on raw data from Petit *et al.* 2014 for comparison with figure 5.2). That is 8 % higher than the heat production capacity seemingly guaranteeing maximal within-winter survival in our population. One can

wonder then why would birds maintain a winter Msum as high as 1.58 W if it does not further improves chances of survival and thus fitness? One hypothesis is that, since upregulating muscle size for endurance exercise also leads to an increase in thermogenic capacity (Swanson, 1995; Swanson & Dean, 1999; Vézina *et al.*, 2007), chickadees could maintain large flight muscles in the winter primarily for active foraging to maximize food intake at the coldest time of the year while the increase in thermogenic performance would in fact be an added benefit. Comparing birds experimentally trained for active exercise and cold would be one way to test this hypothesis (Zhang *et al*, 2015).

High winter thermogenic capacity does not guarantee long-term survival

Our results revealed that survival among years was not related to individual thermogenic capacity or any of our other physiological parameters. Given that birds adjust their phenotype ahead of the peak of cold, increasing Msum for the current season may improve an individual's immediate survival but seems not to guarantee survival among years. Although Msum can be repeatable across years (Cortés *et al.*, 2015), it is nevertheless a highly flexible parameter (Swanson, 2010; Swanson & Vézina, 2015) which, at the population scale, may differ between winters according to minimal ambient temperatures (Petit *et al.*, 2013; Swanson & Olmstead, 1999). Adjusting thermogenic capacity to a particular set of wintering conditions may therefore have little influence on long-term survival. Furthermore, other costly life-history stages, such as breeding or molt, also come with physiological constraints that may influence fitness (Jacobs & Wingfield, 2000). Physiologically constraining events happening in the spring or summer may therefore affect individual condition and bear fitness consequences carrying over to the next winter (Harrison *et al.*, 2011). This could prevent survival analyses from detecting effects of Msum measured in a particular winter on survival over several years.

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CONCLUSION GÉNÉRALE

Les changements climatiques actuels sont caractérisés par une augmentation de la stochasticité des conditions météorologiques (IPCC, 2014), notamment en hiver dans les régions tempérées froides (DesJarlais *et al.*, 2010). Les individus incapables d'ajuster leur phénotype aux fluctuations environnementales n'auraient d'autres choix que de se déplacer vers d'autres habitats pour survivre (Canale & Henry, 2010; Reed *et al.*, 2010; Van De Pol *et al.*, 2010). Par conséquent, la dynamique des populations résidant dans ces régions dépendrait, entre autre, de leur capacité à répondre aux changements environnementaux à court terme *via* la flexibilité phénotypique (Canale & Henry, 2010), *i.e.* la capacité d'un animal à ajuster de façon rapide et réversible son phénotype aux variations de l'environnement (Piersma & Drent, 2003). Il est donc essentiel d'étudier la capacité des individus à ajuster leur physiologie pour comprendre l'effet des variations environnementales sur la valeur sélective, sur la dynamique des populations et sur le devenir des espèces.

L'hiver sous les hautes latitudes est généralement considéré comme une saison énergétiquement difficile pour les espèces endothermes (Chappell, 1980; Cooper, 2000) puisque qu'elles doivent augmenter la part de leur budget énergétique consacrée à la thermogénèse (Liknes & Swanson, 1996) alors que l'accès à la nourriture est limité (Swanson, 2010). Pour faire face à ces conditions contraignantes, les espèces résidentes ajustent leur phénotype, c'est l'acclimatation hivernale. L'acclimatation est surtout étudiée chez les oiseaux de petite taille car non seulement, à masse égale, les oiseaux dépensent plus d'énergie que les mammifères mais aussi parce que, du fait de leur rapport surface/volume élevé, les petites espèces sont plus sensibles au froid que les grandes espèces (McNab, 1971). Ainsi, en comparant des oiseaux maintenant un phénotype d'été à des oiseaux maintenant un phénotype d'hiver (Cooper, 2002; Cooper & Swanson, 1994; Liknes & Swanson, 1996; Swanson, 1991a; Zheng *et al.*, 2008), les études sur l'acclimatation hivernale ont révélé que la plupart des espèces résidant

sous les hautes latitudes augmentaient leur prise alimentaire (McWilliams *et al.*, 1999; Vézina *et al.*, 2011), leur masse corporelle et leurs réserves lipidiques (Cooper, 2002; Zheng *et al.*, 2008), la taille de leurs organes (Liknes & Swanson, 2011b; Zheng *et al.*, 2008) et de leurs muscles (Cooper, 2002; Liknes & Swanson, 2011b) ainsi que leur métabolisme de base (BMR) et leur capacité thermogénique (Msum) (Cooper & Swanson, 1994). Ces études suggèrent donc que, pour optimiser leur survie, les oiseaux de petite taille répondent aux froides températures hivernales en augmentant la taille et l'activité de leurs muscles et de leurs organes, ce qui augmenterait leur métabolisme de base et améliorerait leur capacité thermogénique. Bien que ces études renseignent sur la capacité des oiseaux à ajuster leur phénotype à différents environnements thermiques, elles négligent néanmoins plusieurs éléments. Par exemple :

1) Les oiseaux expriment un métabolisme hivernal plus élevé qu'en été mais ont-ils la capacité d'ajuster leur phénotype au sein de l'hiver afin de suivre les fluctuations des conditions météorologiques ?
2) Les oiseaux ajustent leur phénotype en fonction de la température mais comment répondent-ils aux autres facteurs météorologiques?
3) L'augmentation de la capacité thermogénique est supposée être due à l'augmentation de taille des muscles mais cette relation reste à démontrer expérimentalement.
4) Le métabolisme est censé être lié à la taille des organes et des muscles mais quelle est la contribution relative de chaque tissu à la performance métabolique?
5) L'augmentation de la performance métabolique hivernale est supposée conférer une meilleure survie aux oiseaux résidant en milieux froids mais cette relation reste à démontrer.

Afin de compléter nos connaissances sur la capacité des oiseaux à répondre aux variations thermiques, cette thèse utilise comme modèle une population de mésanges à tête noire en conditions naturelles pour analyser: le patron intra-hivernal des ajustements métaboliques (***chapitre 1***), l'effet des conditions météorologiques (*i.e.* cause exogène, ***chapitre 2***) et de la composition corporelle (*i.e.* mécanisme endogène, ***chapitres 3 et 4***) sur ces ajustements et l'effet de ces ajustements (*i.e.* conséquence, ***chapitre 5***) sur la survie.

Pour la suite de la conclusion générale, les parties C.1 à C.4 récapitulent les principaux résultats de chaque chapitre alors que la partie C.5 discute de l'ensemble des résultats dans un contexte général.

C.1 Ajustements de la performance métabolique au sein de l'hiver

Avec le ***chapitre 1***, nous avons suivi les variations de la performance métabolique au niveau populationnel et individuel durant deux hivers consécutifs. Cette étude a mis en évidence une légère augmentation (+6%) du BMR entre le début de l'hiver en octobre et le pic de froid en février. Puis, en mars, une fois le pic de l'hiver passé, nous avons observé une réduction (-6%) des coûts de maintenance jusqu'à une valeur de BMR similaire à celle de l'été alors que les températures ambiantes étaient encore froides (température minimale au mois de mars : 2010 = -16°C, 2011 : -27°C). Ces résultats suggèrent que les mésanges ajustent leur BMR sur une courte échelle de temps (*i.e.* < 1 mois) au sein de l'hiver mais que la température ambiante n'est probablement pas le facteur environnemental qui influence majoritairement le BMR hivernal des mésanges.

En ce qui concerne la capacité thermogénique maximale, en moyenne, le Msum augmentait graduellement (+25%) entre le début de l'hiver et le pic de froid pour légèrement diminuer (-5%) en mars. Cependant, le patron d'ajustement du Msum différait entre les deux années de l'étude. Alors que la première année, le Msum atteignait sa valeur maximale au mois de février pour décliner légèrement en mars, la deuxième année, c'est au mois de mars que le Msum était le plus élevé. D'après les données de température, le deuxième hiver fut plus long et plus froid que le premier. Ainsi, pour faire face au mois de mars encore très froid de la deuxième année, les mésanges auraient maintenu leur capacité thermogénique à un niveau élevé. Ces résultats révèlent que les mésanges sont capables d'ajuster leur capacité thermogénique relativement rapidement (*i.e.* < 1 mois) et supportent l'idée que la température ambiante minimale aurait une influence majeure sur la capacité thermogénique hivernale des mésanges (Swanson & Olmstead, 1999).

Bien qu'ils aient suivi le même patron d'ajustement, le BMR et le Msum, après correction pour la masse corporelle, n'étaient pas corrélés, ce qui implique un découplage fonctionnel

entre les deux paramètres. De plus, alors que le BMR ne commençait à augmenter qu'au mois de décembre, le Msum avait déjà, au mois d'octobre, une valeur 22% supérieure à sa valeur d'été. Ce décalage temporel entre le début de l'augmentation du BMR (décembre) et celui du Msum (avant octobre) appuie l'hypothèse selon laquelle le métabolisme de base et la capacité thermogénique répondraient à des facteurs environnementaux différents mais variant de concert durant l'hiver (Vézina *et al.*, 2010). Une hypothèse est que les premières chutes de neige importantes de décembre entraîneraient une diminution de l'accès à la nourriture. Dans ces conditions de restriction alimentaire, les oiseaux accélèreraient leur prise alimentaire en augmentant la masse de leurs organes digestifs, et donc de leur BMR (mais voir ***section C.3***). Quant au Msum, il augmenterait surtout en réponse aux froides températures nocturnes qui débutent avec la fin de l'été.

Cette étude a aussi révélé que les ajustements métaboliques de la population reflétaient les ajustements métaboliques intra-individuels, démontrant une certaine flexibilité métabolique chez les mésanges. Ainsi, en ajustant relativement rapidement leur métabolisme, les mésanges à tête noire sont capables, dans une certaine mesure, de survivre à la variation thermique de leur environnement.

C.2 Facteurs météorologiques liés aux variations métaboliques

Avec le ***chapitre 2*** nous avons analysé les variations du BMR et du Msum des mésanges en fonction d'un gradient de conditions météorologiques. Les résultats ont montré que les mésanges à tête noire augmentaient leur BMR quand la température ambiante diminuait. Le fait que la relation entre le BMR et la température soit linéaire signifie, qu'en dépit du large intervalle de températures (-27°C à +32°C) rencontrées dans notre région, les mésanges n'exprimaient pas leur BMR minimal ou maximal et donc n'atteignaient pas les limites de leur flexibilité pour ce paramètre. Il en ressort que, chez la mésange à tête noire, les coûts de maintenance ne sont que partiellement influencés par la température. Nos résultats ont aussi révélé que la variation du BMR était en partie expliquée par une différence inter-individuelle, avec une répétabilité de 0,20. Donc, au sein de la population étudiée, certains individus exprimaient un métabolisme de base constamment plus élevé que leurs congénères, ce qui

pourrait impliquer des différences de stratégie individuelle en termes d'investissement dans la maintenance physiologique (Brodin, 2007).

En ce qui concerne le lien entre les conditions météorologiques et la capacité thermogénique, nos résultats ont mis en évidence un effet combiné de la température et de l'humidité absolue sur le Msum. La norme de réaction du Msum en fonction de la température ambiante suivait une courbe sigmoïde avec un Msum minimal moyen (1,23 W) atteint à 24°C et un Msum maximal moyen (1,55 W) atteint à -10°C. Entre ces deux températures, la capacité thermogénique maximale augmentait linéairement avec la diminution de la température ambiante. D'après les données météorologiques enregistrées à notre site d'étude, dès le mois d'août, la température minimale moyenne se maintient en-dessous de 24°C. Conséquemment, la norme de réaction du Msum et les données de températures minimales impliquent que dès la fin de l'été, alors que la température moyenne est encore relativement chaude, les mésanges commencent déjà à augmenter leur capacité thermogénique en prévision des futures températures hivernales. Ceci supporte les résultats de notre étude précédente sur le patron d'ajustement de la performance métabolique (***chapitre 1***) où nous montrions que les mésanges exprimaient une capacité thermogénique déjà 22% plus élevée en octobre qu'en août. En supposant que la norme de réaction du Msum soit généralisable à toutes les populations de mésanges à tête noire, nos résultats suggèrent que les mésanges des populations plus nordiques, telles que les populations d'Alaska, devraient toujours maintenir une capacité thermogénique élevée puisque la température moyenne en été est généralement inférieure à 20°C. Ces oiseaux seraient donc obligés de maintenir un phénotype hivernal tout au long de l'année, même en été.

Nos observations revèlent que les mésanges de notre population exprimaient leur Msum maximal moyen (1,55 W) dès que la température minimale atteignait -10°C, une valeur correspondant à la température minimale moyenne au pic de l'hiver dans l'aire d'étude (jan: −11,2°C; fév: −11,5°C). Ainsi, durant les mois les plus froids de l'hiver, et ce malgré de nombreux pics de froid à des températures inférieures à -20°C, les mésanges exprimaient une capacité thermogénique maximale ajustée à leur environnement thermique moyen et non aux températures extrêmes qu'elles rencontraient. Le fait que les mésanges n'augmentaient plus leur Msum pour des températures minimales inférieures à -10°C pourrait supposer que leur capacité à

produire de la chaleur est limitée physiologiquement et/ou morphologiquement (McKechnie, 2008). Par exemple, les muscles pectoraux, principal site de la thermogénèse par frissonnement, pourraient être à leur taille maximale au pic de l'hiver empêchant toute production de chaleur supplémentaire. Pour faire face aux températures inférieures à -10°C, les oiseaux devraient alors utiliser d'autres stratégies, telles que la sélection de micro-habitats (Wolf & Walsberg, 1996) ou l'hypothermie volontaire (Lewden *et al.*, 2014), afin de compenser le manque de production de chaleur endogène. D'un autre côté, le Msum n'étant qu'un indicateur de la tolérance au froid (Swanson, 2001), le fait que les mésanges maintiennent un Msum stable sous -10°C pourrait signifier que la capacité thermogénique exprimée par les oiseaux à -10°C leur suffit pour résister sur de courtes périodes à des températures plus froides (*e.g.* -20°C). Dans ce cas, les oiseaux utiliseraient donc la stratégie de la capacité de réserve (Hammond & Diamond, 1992) pour ajuster leur capacité thermogénique durant la période la plus froide de l'hiver. C'est-à-dire que les mésanges ajusteraient leur capacité thermogénique à une valeur stable mais plus élevée que ce que requiert leur environnement. L'avantage étant que les mésanges exprimeraient toujours une tolérance au froid leur permettant d'affronter les températures hivernales. L'inconvénient étant que les mésanges dépensent plus d'énergie que nécessaire dans un environnement énergétiquement contraignant.

La capacité thermogénique des mésanges était aussi affectée par l'humidité absolue selon une courbe en U, avec un Msum minimal atteint à une humidité de 2,8 $g.m^{-3}$. Pour une température donnée, la production de chaleur des mésanges était donc plus élevée à la fois lorsque la proportion d'eau dans l'air diminuait et augmentait. L'air sec favorisant l'évapotranspiration et les pertes de chaleur corporelle, il n'est pas surprenant de constater que, pour une température donnée, les mésanges augmentaient leur Msum avec la diminution de l'humidité dans l'air. A l'opposé, l'humidité de l'air n'est censée avoir qu'un effet négligeable sur les pertes de chaleur à basses températures à cause de la condensation de l'eau dans l'air. Nous avons donc été surpris d'observer qu'au-dessus d'une humidité de 2,8 $g.m^{-3}$, la capacité thermogénique des mésanges augmentait aussi. Toutefois, il est à noter que 35% de nos données furent obtenues à des températures supérieures à 0°C mais inférieures à la température critique minimale (LCT) de la mésange à tête noire (16°C en hiver et 19,9°C en été; Cooper & Swanson, 1994). Une explication serait donc que, dans cette gamme de températures (*i.e.* au-dessus de 0°C mais sous la LCT), l'augmentation de la quantité de vapeur d'eau dans l'air entraine une augmentation des

pertes de chaleur corporelle par conduction à laquelle les mésanges répondraient en augmentant leur capacité thermogénique.

Enfin, nos analyses ont aussi montré que, contrairement au BMR, la variation du Msum n'était pas liée à une différence individuelle. Pour une masse donnée, tous les oiseaux exprimaient donc une capacité thermogénique similaire et une flexibilité d'ajustement comparable. La capacité thermogénique maximale étant supposément liée à la survie des individus (Jackson *et al.*, 2001), le fait que le Msum ne variait pas entre les individus suggère que la capacité thermogénique serait sous une pression de sélection stabilisante qui réduirait la diversité des phénotypes. Ainsi, les individus avec une capacité thermogénique trop faible ne pourraient survivre aux vagues de froid qui caractérisent l'hiver sous les hautes latitudes alors que les individus maintenant une capacité thermogénique trop élevée le feraient au détriment d'autres activités liées à la valeur sélective telle que les défenses immunitaires (Eraud *et al.*, 2005) ou antioxydantes (Costantini & Dell'Omo, 2006). Une étude effectuée sur la même population de mésanges à tête noire sur plusieurs années a montré que la répétabilité intra-hivernale du Msum différait entre les années, passant de non significative (comme l'étude présente) à une valeur significative d'environ 0,40 (Cortés *et al.*, 2015). La répétabilité du Msum au sein de l'hiver est donc dépendante de l'année, ce qui implique que la variation inter-individuelle du Msum dépendrait des conditions hivernales et que la sélection ne jouerait un rôle stabilisant que sous certaines conditions environnementales (*e.g.* température hivernale très froide). Alternativement, le Msum exprimé par les mésanges de notre étude pourrait être si variable au sein d'un même individu qu'il nous aurait été impossible de détecter la moindre répétabilité dans le Msum des mésanges et donc de mettre en évidence une différence de capacité thermogénique entre les individus.

C.3 Contribution de la composition corporelle aux ajustements métaboliques

Avec ***les chapitres 3 et 4*** nous avons étudié la relation entre la masse des organes et des muscles et la performance métabolique chez une population naturelle de mésanges à tête noire résidant en région tempérée froide. Dans un premier temps (***chapitre 3***), nous avons diminué la

surface de portance des ailes et de la queue de certains oiseaux pour les forcer à accroître la taille de leurs muscles pectoraux, ce qui devait conduire à une augmentation de leur capacité thermogénique. Avec cette étude expérimentale, nous avons mis en évidence un lien de cause à effet entre la taille des muscles pectoraux et la capacité thermogénique maximale. En effet, nos résultats ont montré que l'augmentation de la masse des muscles pectoraux engendrait une hausse de la capacité thermogénique chez la mésange à tête noire. Cependant, cette relation était non linéaire et que les mésanges aient de gros muscles (indice 3) ou des muscles de taille moyenne (indice 2), elles exprimaient le même Msum. Ceci suggère que l'augmentation de la taille de muscles du niveau 2 au niveau 3 compense la réduction de surface de portance et permet aux oiseaux de maintenir leur capacité de vol sans pour autant leur conférer une meilleure capacité thermogénique.

Cette étude expérimentale a aussi révélé une relation quadratique entre l'hématocrite, un indicateur de la capacité de transport de l'oxygène, et la capacité thermogénique, avec un Msum maximal atteint pour un hématocrite de 0,53. Ce résultat supporte l'hypothèse selon laquelle, au-dessus d'un certain seuil, le bénéfice d'augmenter la capacité de transport de l'oxygène en augmentant la proportion d'hématies dans le sang est contrebalancé par l'augmentation de la viscosité du sang (Schuler *et al.*, 2010; Williams *et al.*, 2012). Ainsi, les mésanges à tête noire augmenteraient leur capacité thermogénique avec leur capacité de transport de l'oxygène dans le sang, jusqu'à un certain niveau (0,53). Passé ce seuil, les inconvénients d'avoir un hématocrite élevé (*e.g.* augmentation de la viscosité du sang) empêcheraient les mésanges d'augmenter davantage leur capacité thermogénique maximale.

Dans un deuxième temps (***chapitre 4***), nous avons analysé la relation entre la composition corporelle, les coûts de maintenance physiologique (BMR) et la capacité thermogénique (Msum) au cours de l'année. En ce qui concerne les ajustements morphologiques au cours de l'année, nos résultats ont montré que les mésanges augmentaient leur masse maigre sèche et leur masse lipidique entre l'été et l'hiver. Nous avons aussi constaté que la masse des organes excréteurs (foie+reins) ne variait pas au cours de l'année alors que la masse des organes digestifs (intestin+gésier) diminuait entre l'automne et l'été. Nous avons également observé que la masse des organes cardio-pulmonaires (cœur+poumons) et celle des muscles augmentait entre l'automne et le pic de l'hiver pour diminuer entre la fin de l'hiver et l'été.

Pour ce qui est de la contribution relative de la masse des tissus à la variation du BMR et du Msum, nous avons trouvé une relation entre le BMR et les organes excréteurs sur l'année et au pic de l'hiver mais pas aux autres saisons. Le foie peut être considéré comme un organe thermogénique (Baconnier *et al.*, 1979; Vézina *et al.*, 2006; Villarin *et al.*, 2003; Zheng *et al.*, 2013a) et l'augmentation hivernale de la production active de chaleur pourrait donc être due, au moins en partie, à l'augmentation de l'activité métabolique du foie en hiver. Ainsi, bien que nous n'ayons pas observé de variation significative de la masse du foie au cours de l'année, l'augmentation de l'intensité métabolique du foie au pic de l'hiver pourrait engendrer une hausse des dépenses énergétiques associées à la maintenance du foie, ce qui résulterait en une augmentation du BMR. Nous avons aussi mis en évidence une relation entre le BMR et les organes digestifs au pic de l'hiver. Cependant, au pic de l'hiver, la contribution des organes digestifs au BMR n'était pas significative. L'« hypothèse de la demande accrue » propose que l'augmentation du BMR en hiver est due à une augmentation de la prise alimentaire menant à un accroissement de la taille des organes digestifs et donc des coûts de maintenance (*i.e.* « increased demand hypothesis », Liknes & Swanson, 2011b; Liu & Li, 2006; Williams & Tieleman, 2000; Zheng *et al.*, 2013a). Or, nos résultats suggèrent que, bien que la masse des organes digestifs soit 46% plus importante au pic de l'hiver qu'en été, le BMR hivernal ne dépendrait que faiblement de la masse des organes digestifs dans la population étudiée. D'après notre analyse, c'est la masse des muscles squelettiques qui influençait principalement le BMR sur l'année, en automne et à la fin de l'hiver. Les muscles consommant peu d'énergie au repos (Scott & Evans, 1992), il peut sembler surprenant de trouver une relation entre la masse des muscles et le BMR. Cependant, la masse maigre sèche des muscles squelettiques représentait 64% de la masse maigre sèche totale des mésanges à tête noire. Il est donc logique que la maintenance des muscles squelettiques engendrent des dépenses énergétiques importantes et donc que la masse des muscles au repos contribuent majoritairement aux coûts de maintenance.

Nos résultats ont également montré que la capacité thermogénique était principalement dépendante de la masse des muscles squelettiques sur l'année, au pic et à la fin de l'hiver. Combiné à l'effet des muscles, nous avons aussi détecté un effet sur l'année des organes cardio-pulmonaires sur le Msum. Globalement, nos résultats supportent les observations présentées au ***chapitre 3*** et l'idée que la production de chaleur maximale chez les oiseaux

dépende principalement de la capacité de frissonnement et donc de la masse de leurs muscles pectoraux (Cooper, 2002; Saarela & Hohtola, 2003; Swanson *et al.*, 2013; Vézina *et al.*, 2007). Ils démontrent aussi que la capacité thermogénique est affectée par la masse du cœur et des poumons (Swanson *et al.*, 2014a) et donc la capacité à approvisionner l'organisme en oxygène (Swanson, 1990b).

C.4 Conséquences des ajustements métaboliques hivernaux sur la survie

Avec le ***chapitre 5*** nous avons étudié les conséquences des ajustements hivernaux sur la survie d'une population de mésanges à tête noire en conditions naturelles. Nos résultats ont montré que la survie intra-hivernale n'était pas liée à la masse corrigée pour la taille, ni au BMR ou à l'hématocrite des individus. Ainsi, l'augmentation hivernale de la masse, du BMR (***chapitre 1***) ou de l'hématocrite (***chapitre 3***) seule ne contribuerait pas à améliorer la survie hivernale des mésanges à tête noire. Cependant, la survie intra-hivernale variait avec la capacité thermogénique des mésanges suivant une courbe sigmoïde. En effet, cette étude a montré que les individus exprimant un Msum inférieur à 1,26 W avaient une probabilité de survie inférieure à 50% alors que les individus avec un Msum supérieur à 1,46 W avaient une probabilité de survie de 100%. Les études précédentes nous ont appris que les mésanges avaient un Msum moyen en été de 1,26 W (données non publiées) et qu'elles augmentaient leur capacité thermogénique dès la fin de l'été pour atteindre un Msum maximal de 1,58 W en février (données non publiées). Combinés, ces résultats suggèrent que les mésanges à tête noire ont peu de chance de survivre à l'hiver avec leur capacité thermogénique estivale et qu'elles sont donc obligées d'augmenter leur capacité thermogénique dès les premiers froids, ce qui est supporté par les résultats des ***chapitres 1 et 2***.

L'augmentation du métabolisme entraine une hausse de la production de molécules réactives de l'oxygène (Barja, 1999; Hulbert *et al.*, 2007; Murphy, 2009) délétères pour la survie (Hulbert *et al.*, 2007; Martin & Grotewiel, 2006). Nous pouvons alors nous demander pourquoi les mésanges augmentent leur Msum jusqu'à une valeur de 1,58 W au pic de l'hiver alors qu'un Msum de 1,46 W suffit à leur conférer une probabilité de survie de 100%. Les muscles pectoraux, qui représentent la plus grande partie des muscles squelettiques,

supportent à la fois le vol et la thermogénèse chez les oiseaux. Ainsi, une hypothèse pour expliquer le Msum élevé des mésanges en hiver pourrait être, qu'en plus d'augmenter la masse de leurs muscles squelettiques en hiver pour répondre à leurs besoins thermogéniques, les mésanges augmenteraient aussi la masse de leurs muscles pectoraux afin de compenser la surcharge due à l'augmentation de leurs réserves lipidiques et maintenir leur manœuvrabilité (Brodin, 2007). Cette hypothèse est supportée par nos résultats du ***chapitre 3*** où nous montrions que les oiseaux possédant les plus gros muscles pectoraux avaient une capacité thermogénique similaire aux individus avec une taille de muscle moyenne. L'augmentation de la capacité thermogénique chez nos mésanges entre décembre et février (***chapitres 1 à 4***) jusqu'à une valeur supérieure à ce qui est requis pour assurer la survie de la population (> 1,46 W) serait donc possiblement liée à la capacité de vol des individus.

Finalement, cette étude n'a montré aucun lien entre le phénotype hivernal et la survie inter-annuelle. Ces résultats suggèrent donc qu'en dépit du bénéfice d'augmenter la capacité thermogénique pour la survie hivernale, augmenter sa masse, sa capacité de transport de l'oxygène ou sa performance métabolique en hiver n'a pas d'impact sur la survie à long terme. Bien qu'une étude sur la même population de mésanges ait montré que le Msum pouvait être répétable entre les années (Cortés *et al.*, 2015), le Msum est un paramètre très flexible (Swanson, 2010; Swanson & Vézina, 2015) qui, à l'échelle de la population, peut varier d'une année à l'autre en fonction de la température ambiante (Swanson & Olmstead, 1999). Ainsi, l'ajustement de la capacité thermogénique à des conditions hivernales spécifiques pourrait n'avoir que peu d'influence sur la survie à long terme, d'autant plus que d'autres stades d'histoire de vie énergétiquement coûteux (*e.g.* reproduction) peuvent aussi engendrer des coûts physiologiques réduisant la condition des individus, et par conséquent, la survie à long terme (Jacobs & Wingfield, 2000).

C.5 Synthèse

L'augmentation de la stochasticité des conditions météorologiques prévues pour les prochaines années (IPCC, 2014) pourrait entrainer la disparition d'espèces incapables d'ajuster efficacement leur physiologie aux variations thermiques de leur environnement

(Canale & Henry, 2010). Pour prévoir la dynamique des populations (*e.g.* densité, distribution, démographie), il est donc primordial de comprendre l'effet des variations météorologiques sur la condition physiologique des individus et les réponses physiologiques qu'ils mettent en place pour assurer leur survie. C'est avec cet objectif que cette thèse visait à mettre en évidence la capacité des mésanges à tête noire, un oiseau de petite taille résidant en région tempérée froide, à ajuster leur physiologie dans un environnement thermique hivernal variable.

Pour ce projet, nous nous sommes surtout focalisés sur les ajustements de deux paramètres métaboliques, le métabolisme de base (BMR) et la capacité thermogénique maximale (Msum). Le BMR est un indicateur de l'énergie minimale nécessaire à un individu pour assurer le fonctionnement de son organisme alors que le Msum est une mesure de la production maximale de chaleur et est considérée comme un indicateur de la résistance au froid. Jusqu'à maintenant, nos connaissances sur la flexibilité du BMR et du Msum chez des populations des régions nordiques se limitaient principalement au fait que, généralement, les oiseaux de petite taille augmentent leur BMR et leur Msum entre l'été et l'hiver (Cooper, 2002; Liknes *et al.*, 2002; Zheng *et al.*, 2008) en réponse aux froides températures (Swanson & Olmstead, 1999). La hausse du BMR étant principalement associée à une augmentation de la masse des organes digestifs (*e.g.* gésier, intestins, Liknes & Swanson, 2011b; Liu & Li, 2006; Zheng *et al.*, 2008 mais voir Chappell *et al.*, 1999) et celle du Msum étant surtout liée à un accroissement de la taille des muscles et de l'activité des enzymes du métabolisme aérobie (Liknes & Swanson, 2011a; Swanson *et al.*, 2013; Zheng *et al.*, 2008). Bien que ces études nous renseignent sur la capacité des oiseaux à répondre au froid, elles apportent cependant peu d'informations sur la capacité des oiseaux à ajuster rapidement leur métabolisme aux variations à court terme de leur environnement thermique.

C.5.1 Les ajustements du métabolisme de base

En hiver, avec la diminution de la durée du jour et l'augmentation du couvert neigeux, l'accès à la nourriture est restreint par rapport à l'été. Dans ces conditions, pour acquérir assez de nourriture et maintenir un budget énergétique équilibré, les individus pourraient augmenter leur consommation de nourriture à chaque épisode d'alimentation, ce qui aurait

pour conséquence d'augmenter la masse de leurs organes digestifs et ainsi leur métabolisme de base (Liknes & Swanson, 2011b; Liu & Li, 2006; Zheng *et al.*, 2008). En parallèle, pour supporter une hausse d'activité locomotrice associée à l'augmentation de leur quête alimentaire et pour maintenir leur manœuvrabilité en dépit de l'accumulation des réserves lipidiques, les oiseaux pourraient également augmenter la masse de leurs muscles squelettiques (Dietz *et al.*, 2007). Or, un gain de masse musculaire non seulement améliore la capacité de production de chaleur (***chapitres 3 et 4***) (Swanson *et al.*, 2013) mais engendre aussi des coûts de maintenance (Chappell *et al.*, 1999). En théorie donc, en plus d'être lié à la température ambiante (Broggi *et al.*, 2007; Swanson & Olmstead, 1999), le BMR hivernal des oiseaux de petite taille serait aussi dépendant indirectement de l'accès aux ressources alimentaires et de leur capacité de vol. Cependant, nos résultats ne supportent qu'en partie ces deux hypothèses.

Nous avons mis en évidence au ***chapitre 1*** que, en dépit de la diminution de la température ambiante dès la fin de l'été, les mésanges ne commençaient à augmenter leur BMR qu'en décembre, période correspondant au début des chutes importantes de neige. Ceci implique que le BMR serait peu lié à la température mais répondrait plutôt à une limitation de l'accès à la nourriture. Cependant, nous avons aussi observé au ***chapitre 4*** que, entre l'automne et le pic de l'hiver, les mésanges réduisaient la masse de leur système digestif de 10%. De plus, nos analyses démontrent que, bien que les organes digestifs aient été retenus par la sélection de modèle comme variable influençant la variation du BMR au milieu de l'hiver, leur effet n'est pas statistiquement significatif. Ce constat contredit donc l'idée que la variation hivernale du BMR dans notre population soit liée à la masse des organes digestifs et donc à l'accès aux ressources alimentaires. Enfin, au ***chapitre 4***, nous avons remarqué que les mésanges augmentaient la masse de leurs muscles squelettiques de 11% entre l'automne et le pic de l'hiver et que la masse des muscles contribuait majoritairement à la variation du BMR. En résumé, nos résultats suggèrent que, chez les mésanges à tête noire, le BMR est peu affecté par la température *per se* ou par l'accès aux ressources alimentaires et que la hausse hivernale du métabolisme de base chez les oiseaux de petite taille serait plutôt dépendante de l'augmentation de la masse des muscles squelettiques.

Finalement, l'augmentation du BMR en hiver est souvent considérée comme bénéfique pour la survie hivernale (Nzama *et al.*, 2010; Zheng *et al.*, 2008). Or, nos résultats montrent que le BMR hivernal n'est pas relié à la survie des mésanges de notre population. Le BMR représente l'énergie minimale nécessaire pour le fonctionnement de l'ensemble de l'organisme sans renseigner sur l'activité de chacun des tissus. D'un côté, un individu qui occuperait un territoire lui conférant un bon abri aux intempéries, un accès facile à la nourriture et donc une probabilité de survie importante pourrait exprimer des dépenses énergétiques hivernales plus basses que ses congénères et, par conséquent, un BMR relativement bas (Lewden *et al.*, 2012). Alternativement, un tel individu pourrait aussi avoir une activité importante pour défendre son territoire et donc exprimer un BMR élevé (Careau & Garland Jr, 2012; Careau *et al.*, 2008). Il en résulte que la relation entre le BMR et la survie des individus est probablement dépendante du contexte environnemental (Burton *et al.*, 2011; Careau & Garland Jr, 2012). Dans notre cas, bien que le BMR nous renseigne sur l'investissement énergétique des individus dans leur maintenance physiologique, le métabolisme de base des mésanges paraît ne pas être lié à leur valeur sélective. Si l'on considère que la qualité d'un individu est défini par l'ensemble des paramètres qui influencent sa valeur sélective (Wilson & Nussey, 2010), le BMR des oiseaux de petite taille résidant en régions tempérées froides n'est donc apparemment pas un indice de leur qualité.

C.5.2 Les ajustements de la capacité thermogénique

Les mésanges à tête noire ajustent leur capacité thermogénique au sein de l'hiver. Cependant, elles commencent à augmenter leur Msum dès la fin de l'été, alors que la température minimale moyenne est encore clémente (> 15°C). Considérant que les ajustements du Msum dépendent de la taille des muscles (***chapitres 3 et 4***) (Swanson *et al.*, 2013) et que la synthèse des tissus musculaires est un processus relativement lent (Bauchinger & McWilliams, 2010), l'ajustement de la capacité thermogénique *via* une modification de la composition corporelle n'est probablement pas assez rapide (*i.e.* > quelques jours) pour permettre aux mésanges d'ajuster immédiatement la taille de leurs muscles et leur capacité thermogénique aux variations de températures. Cette interprétation est d'ailleurs supportée par une étude de Swanson et Olmstead (1999) qui montre que les

mésanges ajustent leur Msum à la température hivernale avec un délai de 14 à 30 jours. Une hypothèse est donc que les mésanges pallient à ce délai entre la baisse des températures et la hausse de leur Msum en augmentant leur capacité thermogénique dès que la température ambiante commence à diminuer à la fin de l'été, pour se préparer en avance à l'arrivée de l'hiver.

De la même façon, puisqu'augmenter la taille des muscles est un processus relativement lent (Bauchinger & McWilliams, 2010), les mésanges ne devraient pas être en mesure d'ajuster continuellement leur production de chaleur aux conditions météorologiques. Cette hypothèse est supportée par nos résultats du ***chapitre 2***. En effet, la norme de réaction du Msum en fonction de la température minimale nous apporte deux information majeures : 1) les mésanges commencent à augmenter leur Msum dès que la température minimale descend sous 24°C et 2) les mésanges atteignent leur Msum maximal à la température minimale de -10°C, ce qui correspond à la température minimale moyenne au plus froid de l'hiver dans notre aire d'étude. Ainsi, au pic de l'hiver, les mésanges n'ajustent pas leur Msum aux moindres variations de température mais expriment un Msum stable et ajusté à la température moyenne de leur habitat. Toutefois, cette capacité thermogénique maximale semble conférer aux mésanges une tolérance au froid suffisante pour affronter des températures minimales pouvant aller jusqu'à -25°C (température minimale enregistrée dans notre étude). Ces résultats suggèrent donc que les mésanges maintiennent une capacité de réserve en terme de production de chaleur (Hammond & Diamond, 1992). Le concept de capacité de réserve consiste pour un animal à exprimer un phénotype stable mais à une valeur plus élevée que ce qui est requis en moyenne par son habitat afin de pouvoir affronter certaines conditions extrêmes mais de courte durée en dépit d'un phénotype fixe. L'avantage de cette stratégie réside dans le fait que les individus évitent tout délai entre les variations de leur environnement et leur réponse phénotypique. L'inconvénient est que les individus dépensent plus d'énergie pour maintenir un phénotype à un niveau plus élevé que ce que leur habitat moyen requiert. Ainsi, en hiver, les mésanges expriment toujours une tolérance au froid compatible avec les conditions météorologiques mais, en conte partie, elles doivent investir l'énergie nécessaire pour maintenir un Msum élevé.

Notre étude est aussi la première à mettre en évidence un lien positif entre le Msum et la survie hivernale des oiseaux en conditions naturelles. Le Msum hivernal peut donc être considéré comme un indice de la qualité individuelle chez les mésanges. Ce lien entre Msum et survie a déjà été montré chez les petits mammifères (Jackson *et al.*, 2001), ce qui suggère qu'augmenter la capacité thermogénique en hiver pour maximiser la survie hivernale serait un mécanisme commun aux endothermes de petite taille.

En résumé, nous avons montré que les mésanges à tête noire sont capables d'ajuster leur performance métabolique au sein de l'hiver. Nous avons aussi observé que les mésanges semblent maintenir une capacité thermogénique ajustée à leur environnement thermique moyen et non aux extrêmes de températures. De plus, nous avons vu que, dans notre population, les coûts de maintenance physiologique en hiver n'étaient pas liés à la survie des mésanges, contrairement à leur capacité thermogénique. Ces résultats suggèrent que les mésanges à tête noire résidant en régions tempérées froides, et possiblement les autres passereaux de petite taille, seront capables en ajustant leur phénotype hivernal (*e.g.* modification de leur composition corporelle et/ou augmentation de leur capacité thermogénique) de faire face, dans une certaine mesure, à la stochasticité thermique prévue pour les régions à hautes latitudes dans les années à venir.

C.6 Critiques de l'étude

C.6.1 Contexte et originalité

La flexibilité phénotypique permet aux organismes d'ajuster rapidement leur phénotype et confère aux individus une certaine capacité à affronter les fluctuations environnementales (Canale & Henry, 2010). Il est donc important de connaître la capacité de réponse des individus aux variations de leur environnement pour pouvoir comprendre l'effet des changements environnementaux sur la dynamique des populations. Cependant, il n'existe encore que peu d'études sur la flexibilité phénotypique des oiseaux, au niveau individuel et en conditions naturelles. De plus, à notre connaissance, la majorité de ces études ne se concentrent que sur des paramètres de personnalité ou de reproduction mais peu portent sur des paramètres physiologiques (Brommer, 2013; McKechnie, 2008 mais voir par exemple Buehler *et al.*, 2012;

van de Ven *et al.*, 2013 pour des études de la flexibilité de paramètres physiologiques en captivité).

L'originalité de cette thèse provient de l'approche utilisée, à savoir un suivi intra- et inter-annuel d'une population naturelle de mésanges à tête noire résidant en milieu tempéré froid, qui nous a permis d'analyser les causes, les mécanismes et les conséquences des ajustements métaboliques 1) à l'échelle de la population et à l'échelle individuelle et 2) non seulement au sein de l'hiver, une saison énergétiquement difficile pour cette espèce, mais aussi sur toute l'année. Ce protocole nous a permis de mettre en évidence plusieurs nouveautés. Jusqu'à maintenant, la plupart des études sur l'acclimatation hivernale n'ont fait que comparer le métabolisme d'été avec celui de l'hiver, elles ne renseignaient donc pas sur la capacité d'ajustements à court terme des oiseaux. De même, en omettant d'analyser les ajustements intra-individuels, ces études négligeaient les différences de capacité d'ajustements entre les individus (Nussey *et al.*, 2007). Notre étude sur le patron des ajustements métaboliques (***chapitre 1***) est la première à 1) documenter en milieu naturel, avec une base de données aussi vaste, les ajustements métaboliques hivernaux au niveau de la population et de l'individu ; 2) confirmer que les oiseaux sont en effet capables d'ajuster leur métabolisme en fonction des variations thermiques de l'environnement ; 3) montrer que les ajustements métaboliques mesurés à l'échelle de la population reflètent aussi les ajustements individuels.

De la même façon, bien que le froid soit considéré comme la cause principale de l'augmentation du métabolisme hivernal, seules deux études avaient analysé le lien entre le métabolisme et la température hivernale chez des petits passereaux en liberté (Broggi *et al.*, 2007; Swanson & Olmstead, 1999). Notre étude sur la relation entre les conditions météorologiques et le métabolisme (***chapitre 2***) est la première à révéler la norme de réaction du métabolisme en fonction du gradient naturel de plusieurs paramètres météorologiques, et cela tant au niveau de la population que de l'individu. Les normes de réaction apportent d'importantes informations sur les réponses physiologiques utilisées par les individus pour faire face aux variations naturelles de leur habitat. Malheureusement, elles sont encore peu utilisées pour expliquer les ajustements physiologiques chez les endothermes (McKechnie, 2008).

Le lien entre la capacité thermogénique et la taille des muscles pectoraux n'avait encore jamais été démontré expérimentalement. C'est en utilisant un protocole de manipulation de la taille des muscles de vol (***chapitre 3***), que nous avons démontré le lien de cause à effet entre la taille des muscles pectoraux et la capacité thermogénique. Quant au BMR, plusieurs études ont analysé avec des régressions simples le lien entre le métabolisme de base et la taille des organes internes et ont observé une relation entre le BMR et certains organes tels que le foie, les intestins ou les reins (Chappell *et al.*, 1999; Liu & Li, 2006; Williams & Tieleman, 2000). Cependant, en utilisant une approche multivariée pour analyser l'effet de la composition corporelle dans son ensemble sur le métabolisme de base à différentes périodes de l'année (***chapitre 4***), notre étude a mis en évidence non seulement que le BMR était majoritairement lié à la masse des plus gros tissus, à savoir les muscles squelettiques pour notre espèce, mais qu'en plus la relation entre le BMR et la masse des tissus n'était pas constante au cours de l'année.

Finalement, l'augmentation du métabolisme hivernal est supposée favoriser la survie des populations d'oiseaux résidant en régions tempérées froides (Nzama *et al.*, 2010; Sears *et al.*, 2006; Swanson & Olmstead, 1999). Cependant, aucune étude n'a démontré cette relation. Le protocole de capture-marquage-recapture que nous avons appliqué entre septembre 2009 et décembre 2011 (***chapitre 5***) nous a permis de mettre en évidence pour la première fois un effet positif de la capacité thermogénique sur la survie hivernale d'une petite espèce de passereaux résidant en milieu tempéré froid.

C.6.2 Limites

C.6.2.1 Limites du système de capture

Afin de maximiser le nombre d'oiseaux capturés, les sites de capture étaient pourvus de mangeoires continuellement approvisionnées en graines de tournesol. Or, l'accès aux mangeoires permettrait aux mésanges à tête noire d'acquérir jusqu'à 21% de leur apport énergétique quotidien (Brittingham & Temple, 1992). L'accès aux mangeoires pourrait donc potentiellement expliquer la faible augmentation de BMR observée dans notre population entre l'été et l'hiver (+6%) et pourquoi nous n'avons pas trouvé un lien évident entre la masse des organes digestifs et le BMR au pic de l'hiver. En effet, entre l'été et l'hiver, les oiseaux répondraient à la diminution de la qualité/quantité de la nourriture en augmentant la quantité de

nourriture consommée par période d'alimentation ce qui augmenterait la taille de leurs organes digestifs (Geluso & Hayes, 1999; Zheng *et al.*, 2008) et donc leur BMR (Liu & Li, 2006; Zheng *et al.*, 2008). Cependant, dans notre système d'étude, même si la durée du jour était réduite en hiver, l'accès à la nourriture était prévisible de par la présence des mangeoires. Ainsi, les mésanges auraient pu acquérir assez d'énergie pendant la journée en augmentant leur fréquence d'alimentation sans pour autant augmenter la quantité de nourriture consommée par épisode d'alimentation. Une telle stratégie alimentaire pourrait résulter en une hausse relativement faible de la taille des organes digestifs, expliquant le BMR relativement stable observé au cours de l'année et l'absence de relation entre la masse des organes digestifs et les variations de BMR. Néanmoins, la variation saisonnière de BMR obtenue dans notre étude coïncide avec les valeurs trouvées dans la littérature pour d'autres espèces de passereaux non migrateurs (de -4% chez le roselin familier au Michigan, USA, O'Connor, 1995 à +36% chez le moineau friquet au Zhejiang, Chine, Zheng *et al.*, 2008). De plus, le patron annuel de variation des organes digestifs correspond aussi à ce qui a pu être trouvé par d'autres (Liu & Li, 2006; Zheng *et al.*, 2008). Nous pensons donc que même si la présence de mangeoires a pu influencer les ajustements utilisés par les oiseaux, nos résultats sont fiables et représentatif de notre population.

La présence des mangeoires favoriserait aussi la valeur sélective des oiseaux de petite taille (Brittingham & Temple, 1988; Robb *et al.*, 2008). Ainsi, l'accès prévisible à la nourriture pourrait avoir tamponné les variations naturelles de disponibilité alimentaire et amélioré la survie des mésanges. L'accès constant aux mangeoires pourrait donc avoir fait de notre site d'étude un environnement relativement stable et peu contraignant durant tout le projet, ce qui expliquerait que nos mésanges expriment une survie constante au cours des années et durant l'hiver, malgré les pics de froids. Cela dit, tous les oiseaux étant exposés aux mêmes conditions de température, si la présence des mangeoires conduit effectivement à une surestimation de la survie de la population, elle ne devrait avoir que peu d'effet sur le lien que l'on observe entre la survie des mésanges et leur métabolisme.

Une autre limite associée aux captures provient de l'utilisation de trappes avec appât (*i.e.* graines de tournesol). Lors des captures, nous voulions limiter le temps de préhension afin 1) de réduire l'impact de la capture sur la condition des oiseaux et 2) d'effectuer les prises de sang en moins de trois minutes afin de minimiser l'effet de la capture sur le niveau des paramètres

sanguins (Romero & Reed, 2005). Or, lorsque les oiseaux sont capturés avec un filet, non seulement le dérangement du plumage dû au confinement dans le filet en diminue l'isolation et les oiseaux deviennent rapidement hypothermiques mais en plus le temps de démaillage retarde la prise de sang. Pour ces deux raisons, nous avons choisi de capturer les oiseaux à la trappe plutôt qu'au filet en hiver. Néanmoins, l'utilisation des trappes pourrait potentiellement induire un biais d'échantillonnage en favorisant les captures de certains individus de la population. Par exemple, l'utilisation de trappes pourrait engendrer une capture disproportionnée des individus les plus téméraires/explorateurs. Puisque les individus les plus téméraires auraient un métabolisme plus élevé que leur congénères (Careau & Garland Jr, 2012; Careau *et al.*, 2008), nous obtiendrions une surestimation du BMR moyen de notre population. D'un autre côté, les trappes pourraient aussi attirer de manière disproportionnée des individus en moins bonne condition (*i.e.* sous-alimentés) que leurs congénères et qui prendraient donc le risque d'entrer dans les trappes pour s'alimenter (Gorney *et al.*, 1999; Weatherhead & Greenwood, 1981). En supposant que les individus les moins bien nourris expriment un BMR plus bas que leurs congénères, nous obtiendrions une sous-estimation du BMR moyen de notre population. Cependant, nous avions considéré l'effet potentiel des trappes sur l'échantillonnage dans notre protocole et, pour le réduire, nous laissions en permanence des fausses trappes ouvertes sur les sites de capture afin que tous les individus puissent s'habituer à voir ce nouvel objet dans leur habitat et n'aient pas peur d'y entrer pour se nourrir. De plus, une étude récente du lien entre le métabolisme et la personnalité en conditions naturelles chez des oiseaux a montré que la relation entre le BMR et la personnalité des individus n'était au mieux que très faible (Bouwhuis *et al.*, 2014). Nous pensons donc que, dans le cas où l'utilisation de trappes influencerait le type d'individus capturés (téméraires *versus* affamés), l'effet sur les valeurs du métabolisme ne devrait être que faible. De plus, si l'échantillonnage est influencé par l'utilisation de trappes, il est fort probable que ce soit en attirant à la fois les individus les plus téméraires et les individus les plus nécessiteux, ce qui résulterait en un métabolisme moyen représentatif de la population. Enfin, nos résultats du ***chapitre 1 et 2*** suggèrent que tous les individus ajustent leur métabolisme de façon relativement similaire (les courbes individuelles de variation du BMR et du Msum au cours de l'année ainsi que les normes de réaction individuelles du BMR sont parallèles). Par conséquent, en dépit d'un éventuel effet de l'utilisation des trappes sur notre échantillonnage, les

ajustements utilisés par les mésanges que nous avons mis en évidence n'en restent pas moins valides.

C.6.2.2 Limites des recherches en conditions naturelles

Pour ce projet nous avons suivi la physiologie d'individus confrontés aux variations naturelles de leur environnement. Cependant, travailler en milieu naturel génère deux contraintes principales : 1) notre capacité à suivre les individus est limitée par notre succès de recapture et 2) l'interprétation des résultats est parfois difficile du fait qu'on ne peut contrôler *a priori* les facteurs de l'environnement. Réaliser notre projet en milieu contrôlé aurait pu faciliter notre travail. Cependant, les études expérimentales mènent aussi à des conclusions tronquées du fait que les conditions appliquées ne sont qu'une représentation partielle de l'habitat naturel (Nussey *et al.*, 2007). A l'opposé, bien que les études en milieu naturel ne révèlent dans la plupart des cas que des liens de corrélation entre différentes variables, elles permettent d'analyser les ajustements physiologiques dans des conditions biologiquement pertinentes pour les individus (Brommer, 2013). De plus, les problèmes liés au contrôle de l'environnement en milieu naturel peuvent en partie être résolus par les analyses statistiques qui nous permettent de contrôler certains facteurs *a posteriori*.

C.6.2.3 Limites des paramètres physiologiques utilisés

Le BMR est un indicateur de la quantité minimale d'énergie dépensée par un animal inactif (ni en croissance, ni en reproduction, ni en mue, ni en digestion, etc.) pour maintenir le bon fonctionnement de sa physiologie de base. La mesure du BMR n'est donc pas mesurable en milieu naturel et les conditions expérimentales induisent indubitablement un certain niveau de stress chez les individus mesurés. Si l'on considère qu'un individu stressé augmente son métabolisme, le BMR fournit donc une surestimation de l'énergie minimale nécessaire en conditions naturelles à un individu pour maintenir son organisme. Pour limiter l'effet du stress durant les mesures de BMR, nous enregistrions la consommation d'oxygène des oiseaux pendant toute la nuit afin qu'ils s'habituent au système et pour leur donner le temps de se calmer. De plus, nous sélectionnions le BMR comme étant la consommation d'oxygène la plus faible

pendant 10 minutes consécutives sur les 10 à 11 heures d'enregistrement. Ainsi, bien que le BMR soit une valeur théorique, nous pensons que c'est un bon indicateur de la quantité d'énergie minimale dont a besoin un organisme pour survivre et que nos valeurs de BMR sont représentatives des dépenses énergétiques minimales associées à la maintenance de la machinerie métabolique des mésanges à tête noire.

Le Msum est la quantité d'énergie maximale dépensée par un individu pour maintenir sa température corporelle lorsqu'il est exposé au froid. Non seulement le Msum est mesuré en conditions expérimentales mais en plus c'est une mesure de la capacité maximale des individus à produire de la chaleur et non une mesure de la quantité de chaleur réellement produite par les oiseaux au moment de leur capture en milieu naturel. Cependant, il a été démontré que 1) les oiseaux avec les Msum les plus élevés parvenaient à maintenir leur température corporelle à des températures ambiantes plus froides que leurs congénères exprimant un Msum plus faible (Swanson, 1990a) et 2) exposés à une température froide fixe, les oiseaux avec les Msum les plus élevés maintenaient leur température corporelle plus longtemps que leurs congénères exprimant un Msum plus faible (Swanson, 2001). Nous pensons donc que le Msum est un bon indicateur de la résistance au froid des mésanges à tête noire en milieu naturel.

Un autre avantage majeur à utiliser le BMR et le Msum comme indices des dépenses énergétiques minimales et maximales est que ce sont des variables standardisées qui permettent des comparaisons entre les individus ou les populations dans le temps et/ou dans l'espace.

La dépense quotidienne d'énergie (DEE pour « daily energy expenditure»), qui inclut les dépenses énergétiques de toutes les activités effectuées par un individu pendant 24 heures, aurait été une variable très utile pour étudier les ajustements métaboliques utilisés par des oiseaux confrontés à des conditions environnementales contraignantes. Non seulement c'est une variable qui se mesure en conditions naturelles mais en plus, contrairement au BMR et au Msum, la technique de mesure de la DEE ne restreint pas le nombre d'individus mesurables par jour. Cependant, la mesure de la DEE par l'eau doublement marquée nécessite de capturer un individu une première fois pour lui injecter de l'eau marquée par isotopes stables ($^2H_2{}^{18}0$) et lui faire une première prise de sang, de relâcher cet individu dans son environnement et de le recapturer 24 ou 48 heures plus tard pour effectuer une deuxième prise de sang. C'est un protocole difficilement réalisable en milieu naturel où l'on ne peut être sûr de capturer deux fois un même individu en

moins de 48 heures. De plus, effectuer deux prises de sang en si peu de temps pourrait compromettre la condition physiologique d'animaux aussi petits que des mésanges à tête noire. Enfin, l'analyse des ratios isotopiques $^{2}H/^{1}H$ et $^{18}O/^{16}O$ est onéreuse, ce qui rend cette technique peu accessible lorsque l'on dispose d'un large échantillon, tel que celui utilisé pour ce projet.

C.6.2.4 Limites des données

Les tours météorologiques situées au sein de la forêt Macpès nous ont non seulement fourni des données météorologiques spécifiques à notre site d'étude mais elles nous ont aussi permis d'inclure plusieurs paramètres météorologiques dans nos analyses. Cependant, à cause d'un problème technique d'appareillage, nous n'avons pu utiliser les données de rayonnement solaire. Or, vu l'importance de la radiation thermique dans les échanges de chaleur, il aurait été très pertinent de tester l'effet de ce paramètre sur le métabolisme des mésanges.

De par la nouveauté de notre système d'étude, nous ne disposions de données de survie que depuis septembre 2009, ce qui a limité notre étude sur le lien entre le métabolisme hivernal et la valeur sélective. En effet, pour calculer la probabilité de survie à long terme des mésanges à tête noire, il fallait au minimum avoir recensé la population pendant 3 ans. Les données obtenues fin 2011 nous ont permis de collecter un 3ème hiver pour la cohorte d'oiseaux capturés dès l'automne 2009. Cependant, nous n'avions pas assez de recul pour inclure dans cette analyse la cohorte d'oiseaux capturés pour la première fois à l'automne 2010. Néanmoins, en dépit d'une taille d'échantillon réduite, nous avons tout de même obtenus des résultats clairs quant au lien entre le métabolisme hivernal et la survie à long terme des mésanges.

C.7 Perspectives de recherche

Notre recherche est surtout concentrée sur les ajustements métaboliques sans aborder les autres mécanismes mis en place par les mésanges pour affronter les variations météorologiques. Or, l'augmentation hivernale des ressources énergétiques allouées à la

thermorégulation pourrait entrainer, par exemple, une diminution de l'énergie consacrée aux défenses immunitaires et donc compromettre la valeur sélective individuelle. Donc, pour pouvoir comprendre l'effet des variations thermiques sur la dynamique des populations, il est important d'étudier les ajustements physiologiques dans leur ensemble et de ne pas se limiter à une seule fonction. De plus, nos analyses n'incluent que deux années de données en milieu naturel. Il en résulte quelques incertitudes dans l'interprétation de nos résultats. Par exemple, pour confirmer que les mésanges atteignent leur Msum maximal à -10°C par stratégie et non pas parce qu'elles sont limitées physiquement et ne sont plus capables d'augmenter leur Msum (voir ***chapitre 2***), il faudrait comparer nos données avec un autre jeu de données où la température minimale moyenne serait bien inférieure à -10°C. Par conséquent, cette thèse apporte nombre de nouveaux éléments sur la capacité des oiseaux de petite taille à ajuster leur métabolisme aux variations thermiques de leur habitat ainsi que sur les mécanismes et les conséquences de ces ajustements. Il reste cependant beaucoup d'études complémentaires à effectuer pour améliorer notre compréhension des effets des variations météorologiques sur la physiologie et la dynamique des populations aviaires.

C.7.1 Étude en conditions expérimentales

Au ***chapitre 1***, nous avons proposé que l'augmentation hivernale du BMR et du Msum soit due à différents facteurs: le BMR varie principalement avec l'accès aux ressources alimentaires alors que le Msum dépend surtout des conditions météorologiques. Cependant, puisque nous avons travaillé en milieu naturel, nous n'avons pas pu contrôler séparément ces deux paramètres. Une étude expérimentale combinant deux régimes alimentaires (A: une diète entrainant une augmentation de la taille du système digestif *versus* B: une diète contrôle) et deux traitements thermiques (1: froid *versus* 2: neutre) pourrait venir appuyer cette hypothèse. En théorie, les oiseaux du groupe A1 auraient de gros organes digestifs et donc un BMR élevé ainsi qu'un Msum élevé; les oiseaux du groupe A2 auraient de gros organes digestifs et donc un BMR élevé mais un Msum faible; les oiseaux du groupe B1 auraient de petits organes digestifs et donc un BMR faible mais un Msum élevé; les oiseaux du groupe B2 auraient de petits organes digestifs et donc un BMR faible ainsi qu'un Msum faible. Ce protocole permettrait de mettre en évidence

1) les liens de cause à effets entre la consommation de nourriture et le BMR et la température et le Msum et 2) de dissocier les deux processus.

Au ***chapitre 2***, nous avons proposé que les mésanges expriment une capacité thermogénique maximale ajustée à leur environnement thermique moyen (température minimale de -10°C) et non aux températures extrêmes. Pour confirmer que les mésanges ajustent leur capacité thermogénique à leur environnement moyen par stratégie et non parce qu'elles sont limitées par leur physiologie et/ou leur morphologie, nous pourrions soumettre des mésanges à un environnement moyen de -15°C avec des fluctuations de températures allant de -25°C à -5°C. Les résultats montreraient si les mésanges sont capables d'atteindre un Msum maximum ajusté à l'environnement moyen de -15°C et supérieur à ce que nous avons observé dans notre étude en conditions naturelles.

Au ***chapitre 3***, nous avons mis en évidence une relation quadratique entre la taille des muscles pectoraux et la capacité thermogénique des mésanges. Ceci suggère que, passé une certaine taille, avoir de gros muscles confère une meilleure capacité de vol mais ne permet pas de produire plus de chaleur que d'avoir des muscles de taille moyenne. Une étude expérimentale combinant plusieurs conditions d'activité allant d'un traitement peu actif (*e.g.* petit espace, beaucoup de perchoirs, nourriture et eau facilement accessibles) à un traitement très actif (*e.g.* grand espace, peu de perchoirs, nourriture et eau difficilement accessibles) permettrait d'obtenir des individus avec des tailles de muscles différentes. Des mesures de taille de muscles pectoraux par échographie, de métabolisme maximal induit par le froid (Msum) et de métabolisme maximal induit par l'exercice (MMR) permettraient d'analyser la relation entre la taille des muscles et le Msum et le MMR et de tester l'hypothèse selon laquelle les muscles ont un rendement différent pour le vol et la thermogénèse.

Dans la même étude (***chapitre 3***), nous avons observé une relation non linéaire entre la capacité thermogénique et l'hématocrite, un indicateur de la capacité de transport de l'oxygène dans le sang. Pour confirmer ce lien, nous pourrions manipuler expérimentalement la capacité de transport de l'oxygène dans le sang des mésanges en leur injectant de la phenylhydrazine hydrochloride (PHZ). La PHZ a un effet rapide et réversible et permet de diminuer l'hématocrite (Hct) et l'hémoglobine (Hb) (Williams *et al.*, 2012). Ainsi, en théorie, des individus injectés avec de la PHZ auraient un niveau d'Hct et d'Hb faible et un Msum bas

alors que des individus injectés avec de la saline (= groupe contrôle) aurait un niveau d'Hct et d'Hb normal et un Msum plus élevé que leurs congénères. Une telle étude expérimentale permettrait de tester la relation entre l'oxygénation des tissus et la thermogénèse par frissonnement.

C.7.2 Étude intégrée

Avec cette thèse, nous n'avons étudié qu'une partie des ajustements phénotypiques hivernaux, à savoir les ajustements métaboliques et morphologiques. Or, selon le principe d'allocation des ressources, pour affronter des conditions énergétiquement difficiles, les animaux utiliseraient des compromis physiologiques leur permettant d'allouer plus d'énergie à certaines activités au détriment d'autres (Ardia & Clotfelter, 2007; Schmidt-Wellenburg *et al.*, 2008; Wiersma *et al.*, 2004). Ainsi, en hiver, pour faire face aux conditions environnementales contraignantes, les oiseaux de petite taille pourraient augmenter la part d'énergie consacrée à leur thermorégulation au détriment du maintien de leurs autres activités (*e.g.* système immunitaire, balance oxydative), ce qui pourrait compromettre leur survie hivernale. Pour mieux comprendre l'effet des variations environnementales sur le phénotype hivernal des oiseaux de petite taille, les futures recherches devraient utiliser une approche intégrée pour non seulement analyser les réponses métaboliques mais aussi les ajustements des autres fonctions (*e.g.* système immunitaire) et ainsi mettre en évidence les compromis physiologiques utilisés par les individus pour survivre à l'hiver.

C.7.3 Étude à long terme

Au ***chapitre 2***, nous avons noté que le BMR était variable entre les mésanges et répétable au sein d'une année, ce qui suggère que les individus utilisent des stratégies énergétiques individuelles différentes. Nous supposions alors que les individus qui maintiennent un BMR élevé sont plus performants que leurs congénères et ont une meilleure valeur sélective. Or, au ***chapitre 5***, nous avons observé que la survie des mésanges n'est pas liée à leur BMR, ce qui contredit notre hypothèse. Dans la même étude, nous avons observé un effet positif de la capacité thermogénique sur la survie hivernale des mésanges. Par contre, la capacité

thermogénique des mésanges n'influe pas leur survie à long terme. Il semble donc qu'augmenter la capacité thermogénique hivernale améliore la survie immédiate des oiseaux de petite taille mais pas leur survie à long terme. Néanmoins, les résultats du ***chapitre 5*** n'incluent pas de données de reproduction et ne sont basés que sur trois années de collectes de données, ils sont donc insuffisants pour conclure sur la relation entre la performance métabolique hivernale et la valeur sélective des oiseaux de petite taille résidant en régions tempérées froides. Des recherches en milieu naturel combinant des données de BMR et de Msum avec des données de succès reproducteur et de survie récoltées sur plusieurs années (> 3 ans) permettraient d'analyser si les stratégies énergétiques mises en place en hiver se traduisent par une différence de valeur sélective (*i.e.* survie et succès reproducteur) à long terme entre les individus.

De plus, comme nous l'avons mentionné précédemment, pour affronter l'hiver, les oiseaux de petite taille utilisent des compromis physiologiques mettant en jeu plusieurs fonctions physiologiques (*e.g.* métabolisme, système immunitaire, balance oxydative). Or, à cause des effets reportés (*i.e.* événements se déroulant dans une saison mais ayant des effets sur les saisons suivantes, Fretwell, 1972), les ajustements physiologiques (*e.g.* métaboliques, immunitaires) utilisés par les individus durant la saison hivernale pourraient avoir des répercussions à d'autres moments du cycle annuel (Blais *et al.*, 2001; Hulbert *et al.*, 2007). Par conséquent, pour comprendre l'effet des variations thermiques hivernales sur la dynamique des populations aviaires, il est essentiel de développer des études intégrées et à long terme afin d'analyser les conséquences des ajustements physiologiques hivernaux sur le succès reproducteur et la survie à long terme des individus.

Une autre raison majeure pour utiliser une approche intégrée à long terme est de caractériser la qualité des individus déterminée par l'ensemble des paramètres physiologiques en lien avec leur valeur sélective (figure C.1) (Wilson & Nussey, 2010). En effet, il est par exemple souvent supposé que les oiseaux qui augmentent le plus leur indice de masse corporelle (*i.e.* masse corrigé pour la taille) sont de meilleure qualité que leurs congénères ce qui leur confèrerait une meilleure valeur sélective (Brodin, 2007). Or, nous avons vu au ***chapitre 5*** que, dans notre population, la masse corporelle corrigée par la taille n'était pas liée à la survie des mésanges. Il en ressort que, si le lien entre un trait phénotypique, par exemple la masse corporelle, et la valeur sélective n'a pas été préalablement établie, ce trait ne devrait pas servir d'indicateur de qualité

individuelle. Pour déterminer l'effet des variations thermiques sur la dynamique des populations par l'entremise des ajustements physiologiques, il convient donc d'analyser des paramètres phénotypiques pertinents, c'est-à-dire ceux en lien avec la valeur sélective des individus.

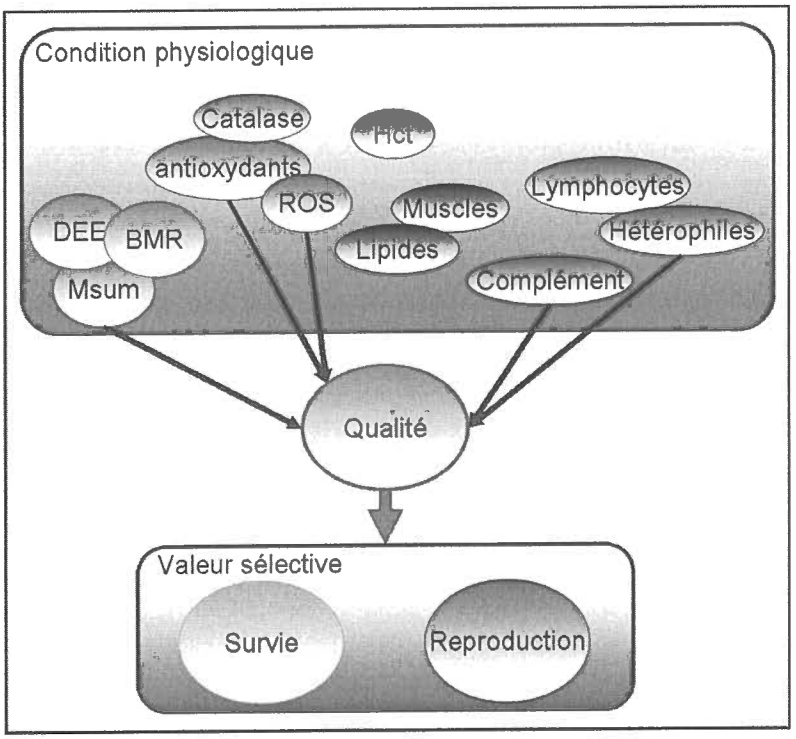

Figure C.1 Condition, qualité et valeur sélective. L'ensemble des paramètres physiologiques détermine la condition physiologique mais seuls certains de ces paramètres caractérisent la qualité individuelle dont dépend la valeur sélective.

C.7.4 Étude en physiologie de la conservation

Les recherches en écophysiologie ont un rôle important à jouer en physiologie de la conservation (Cooke *et al.*, 2013). D'abord, les études en écophysiologie sont nécessaires pour la mise en place de suivis efficaces des populations naturelles. En effet, en analysant les ajustements physiologiques utilisés par les individus en réponse aux variations de leur environnement ainsi que les conséquences de ces ajustements sur leur valeur sélective, les

études en écophysiologie peuvent mettre en évidence les traits phénotypiques qui définissent la qualité des individus. Basés sur ces connaissances, les écologistes de la conservation peuvent alors effectuer des suivis de populations en utilisant des procédures peu ou non invasives (*e.g.* prise de sang) et en choisissant de ne mesurer que quelques paramètres physiologiques parmi les plus pertinents (*i.e.* liés à la survie et/ou à la reproduction). Les études en écophysiologie sont aussi nécessaires pour la mise en place de plans de conservation efficaces à long terme (Cooke *et al.*, 2013). En effet, en mettant en évidence l'effet des variations environnementales sur la qualité des individus, les études en écophysiologie, combinées aux prédictions émanant des études sur les changements globaux, peuvent permettre d'estimer les conséquences des futures perturbations environnementales sur la dynamique des populations. Fortes de ces estimations, les personnes en charge des politiques de conservation (*e.g.* politiciens, gestionnaires de parcs) peuvent alors proposer des stratégies pour limiter les dégradations de l'environnement (*e.g.* modification des techniques de coupes forestières) ou pour favoriser la survie des populations (*e.g.* mise en place de couloirs écologiques).

Un exemple où une étude écophysiologique pourrait être appliquée à des fins de conservation dans notre région concerne la mésange à tête brune (*Poecile hudsonicus*). Il s'agit d'une espèce de mésange boréale très semblable à la mésange à tête noire que l'on trouve aussi sur notre site d'étude. La mésange à tête brune est considérée à « préoccupation mineure » au niveau mondial par l'union internationale pour la conservation de la nature (IUCN) mais la population canadienne, soit 84% de la population mondiale, a connu une diminution importante (> 50%) depuis 1970. Les raisons de ce déclin sont encore mal connues mais seraient potentiellement liées à la dégradation de l'habitat hivernal des mésanges, notamment du fait de l'exploitation forestière (d'après environnement Canada)[1]. Avec une approche intégrée à long terme et en utilisant des procédures peu ou non invasives (*e.g.* collecte de plumes, prise de sang), nous pourrions déterminer 1) les paramètres physiologiques liés à la survie et à la reproduction des mésanges à tête brune et 2) les variables environnementales influençant ces indices de qualité. Une telle étude mettrait en évidence les paramètres de l'environnement liés à la valeur sélective des mésanges à tête

[1] http://www.ec.gc.ca/soc-sbc/oiseau-bird-fra.aspx?sY=2011&sL=f&sM=p1&sB=BOCH

brune et donc permettrait la mise en place de mesures de conservation afin de minimiser la perturbation des paramètres environnementaux impliqués.

ANNEXES

Table A.1 Inter-seasonal variation in body mass, basal metabolic rate (BMR), summit metabolic rate (Msum) and metabolic expansibility (ME). Data are least square means controlling for year, season and sex (and time of capture for body mass) with bird ID as random parameter.

	Body mass (g)	BMR (W)	Msum (W)	ME
Seasons	**Mean ± sem**	**Mean ± sem**	**Mean ± sem**	**Mean ± sem**
Winter	11.44 ± 0.06	0.271 ± 0.002	1.667 ± 0.065	6.19 ± 0.25
Summer	11.42 ± 0.08	0.253 ± 0.004	1.241 ± 0.071	4.99 ± 0.28

Table A.2 Intra-seasonal variation in body mass, basal metabolic rate (BMR), summit metabolic rate (Msum) and metabolic expansibility (ME). Data are least square means controlling for sex, year, month and the interaction year*month for Msum and ME, with bird ID as random parameter. Body mass analysis also included time of capture as covariate.

	Body mass (g)	BMR (W)	Msum (W)/Year 1	Msum (W)/Year 2	ME/Year 1	ME/Year 2
Months	**Mean ± sem**	**Mean ± sem**	**Mean ± sem**	**Mean ± sem**	**Mean ± sem**	**Mean ± sem**
October	11.12 ± 0.08	0.256 ± 0.004		1.316 ± 0.049		5.142 ± 0.200
Novembre	11.03 ± 0.07	0.252 ± 0.004		1.339 ± 0.049		5.268 ± 0.205
December	11.56 ± 0.07	0.264 ± 0.004		1.460 ± 0.049		5.463 ± 0.201
January	11.38 ± 0.06	0.264 ± 0.003	1.483 ± 0.093	1.494 ± 0.049	5.666 ± 0.319	5.840 ± 0.209
February	11.44 ± 0.05	0.271 ± 0.002	1.782 ± 0.080	1.555 ± 0.044	6.549 ± 0.258	5.777 ± 0.187
March	11.35 ± 0.05	0.254 ± 0.003	1.555 ± 0.089	1.572 ± 0.046	6.287 ± 0.305	6.150 ± 0.192
August	11.32 ± 0.07	0.255 ± 0.004	1.274 ± 0.085	1.191 ± 0.057	4.865 ± 0.290	4.856 ± 0.243

Table A.3 Full model explaining mass and sex independent basal metabolic rate (BMR) variation. Residual BMR were extracted from an ANCOVA controlling for body mass and sex. Residual minimal absolute humidity was extracted from a polynomial regression and controls for the effect of ambient temperature.

Full model - N = 243 : AICc = -1119.5

LME fit by REML: ***resBMR ~ poly(Tmin,3) + poly(Pmin,3) + poly(Wmax,3) + poly(resAHmin,3) + (1\|BirdID)***

Random effects

Group Name	Variance	Standard deviation
BirdID (Intercept)	0.00005	0.00699
Residual	0.00020	0.01419

Fixed effects

	Estimate	s.e.m	p-value
(Intercept)	166.00	561.9	0.768
Tmin	0.000	0.000	0.091
$Tmin^2$	0.000	0.000	0.929
$Tmin^3$	0.000	0.000	0.651
Pmin	-4.986	16.93	0.769
$Pmin^2$	0.050	0.170	0.770
$Pmin^3$	0.000	0.001	0.770
Wmax	0.015	0.009	0.085
$Wmaw^2$	-0.007	0.004	0.110
$Wmax^3$	0.001	0.001	0.157
resAHmin	0.001	0.002	0.520
$resAHmin^2$	0.001	0.001	0.537
$resAHmin^3$	0.000	0.000	0.995

Table A.4 Full model explaining mass and sex independent summit metabolic rate (Msum) variation. Residual Msum were extracted from an ANCOVA controlling for body mass and sex. Residual minimal absolute humidity was extracted from a polynomial regression and controls for the effect of ambient temperature.

Full model - N = 222 ; AICc = -115.2			
LME fit by REML: ***resMsum ~ poly(Tmin,3) + poly(Pmin,3) + poly(Wmax,3) + poly(resAHmin,3) + (1|BirdID)***			
Random effects			
Group Name	**Variance**	**Standard deviation**	
BirdID (Intercept)	0.00325	0.057	
Residual	0.01464	0.121	
Fixed effects			
	Estimate	**s.e.m**	**p-value**
(Intercept)	2959.0	5315.0	0.631
Tmin	**-0.017**	**0.002**	**<0.0001**
$Tmin^2$	**0.000**	**0.000**	**<0.0001**
$Tmin^3$	**0.000**	**0.000**	**0.002**
Pmin	-90.28	160.2	0.627
$Pmin^2$	0.918	1.609	0.623
$Pmin^3$	-0.003	0.005	0.620
Wmax	0.017	0.091	0.854
$Wmaw^2$	-0.001	0.042	0.982
$Wmax^3$	-0.001	0.006	0.875
ResAHmin	-0.014	0.018	0.435
$resAHmin^2$	**0.044**	**0.014**	**0.002**
$resAHmin^3$	**0.011**	**0.005**	**0.034**

Table A.5 Basal metabolic rate (BMR) monthly variation. Least square means of BMR were calculated from a linear mixed effect model including body mass and month as fixed parameters and bird ID as random variable.

BMR ~ Month + Mass + (1|BirdID)		
Month: $F_{7,225}$ = 3.3, p< 0.001; Mass:$F_{1,194}$ =123.7, p < 0.01 ; BirdID: df = 1; X^2 = 6.6, p = 0.01		
Month	**Mean (W)**	**s.e.m**
Aug	0.258	0.003
Sep	0.252	0.004
Oct	0.256	0.003
Nov	0.257	0.003
Dec	0.263	0.003
Jan	0.261	0.004
Feb	0.268	0.002
Mar	0.259	0.002

Seasonal increase between August and February = 3.9%

Table A.6 Summit metabolic rate (Msum) monthly variation. Least square means of Msum were calculated from an ANCOVA including body mass and month as fixed parameters.

Msum ~ Month + Mass		
Month: $F_{7,214}$ = 35.7, p< 0.0001; Mass:$F_{1,214}$ =131.5, p < 0.0001		
Month	**Mean (W)**	**s.e.m**
Aug	1.231	0.026
Sep	1.264	0.029
Oct	1.341	0.025
Nov	1.384	0.024
Dec	1.440	0.027
Jan	1.497	0.026
Feb	1.564	0.017
Mar	1.601	0.019

Seasonal increase between August and February = 27.1%

Table A.7 Black-capped chickadees' capture markage recapture (CMR) data collected over three periods during winter 1 for cohort 1 and winter 2 for cohort 2.

#	Cohort	ID	Sep	Oct	Nov	Dec	Jan	Feb	Mar	P1	P2	P3	History
1	1	95008	1	1			1	1	1	1	1	1	111
2	1	95011	1				1		1	1	1	1	111
3	1	95025		1			1		1	1	1	1	111
4	1	95029		1			1		1	1	1	1	111
5	1	95032		1	1		1	1	1	1	1	1	111
6	1	95033		1			1			1	1		110
7	1	95034		1	1		1	1		1	1	1	111
8	1	95036			1			1		1		1	101
9	1	95037			1		1			1	1		110
10	1	95038			1			1	1	1		1	101
11	1	95041			1			1	1	1		1	101
12	1	95061					1		1		1	1	011
13	1	95069						1				1	001
14	1	95097						1				1	001
15	1	95098						1				1	001
16	1	95099						1	1			1	001
17	1	95100						1				1	001
18	1	95105						1	1			1	001
19	1	95109							1			1	001
20	1	95123							1			1	001
21	1	95124							1			1	001
22	1	95125						1	1			1	001
23	1	95127						1				1	001
24	1	95128						1				1	001
25	1	95131						1				1	001
26	1	95132						1				1	001
27	1	95140					1		1		1	1	011
28	1	95142					1	1			1	1	011
29	1	95144					1	1			1	1	011
30	1	95145							1			1	001
31	1	95146					1		1		1	1	011
32	1	95149					1	1			1	1	011
33	1	95150					1	1			1	1	011
34	1	95151					1		1		1	1	011
35	1	95154					1	1			1	1	011
36	1	95155					1	1			1	1	011
37	1	95157					1				1		010
38	1	95159					1	1			1	1	011
39	1	95160					1	1			1	1	011
40	1	95164					1		1		1	1	011
41	1	95165					1	1			1	1	011
42	1	95169					1	1			1	1	011
43	1	95173				1		1			1	1	011
44	1	95174				1	1	1			1	1	011
45	1	95177				1		1			1	1	011
46	1	95182				1	1	1	1		1	1	011
47	1	95184				1	1	1			1	1	011
48	1	95185				1	1				1		010

#	Cohort	ID	Sep	Oct	Nov	Dec	Jan	Feb	Mar	P1	P2	P3	History
49	2	95083		1						1			100
50	2	95085		1	1		1	1	1	1	1	1	111
51	2	95090		1	1		1			1	1		110
52	2	95111	1	1		1				1	1		110
53	2	95113	1	1	1					1			100
54	2	95202		1		1	1	1	1	1	1	1	111
55	2	95208	1							1			100
56	2	95210	1				1	1	1	1	1	1	111
57	2	95213	1		1		1	1		1	1	1	111
58	2	95214		1		1	1	1	1	1	1	1	111
59	2	95215		1	1		1	1	1	1	1	1	111
60	2	95230			1		1	1	1	1	1	1	111
61	2	95259						1	1			1	001
62	2	95263					1	1	1		1	1	011
63	2	95279						1	1			1	001
64	2	95289						1	1			1	001
65	2	95321			1		1	1		1	1	1	111
66	2	95354					1				1		010
67	2	95356					1	1	1		1	1	011
68	2	95358					1	1	1		1	1	011
69	2	95359					1	1	1		1	1	011
70	2	95360					1	1	1		1	1	011
71	2	95362					1	1	1		1	1	011
72	2	95363					1	1	1		1	1	011
73	2	95364					1	1	1		1	1	011
74	2	95365					1				1		010
75	2	95366					1				1		010
76	2	95373						1	1			1	001
77	2	95375						1	1			1	001
78	2	95378						1	1			1	001
79	2	95379							1			1	001
80	2	95390							1			1	001
81	2	95391							1			1	001

P1 = 1 if present between September and November
P2 = 1 if present between December and January
P3 = 1 if present between February and March

Table A.8 Black-capped chickadees' capture-markage-recapture (CMR) data collected during seven periods over three years for cohort 1.

#	ID	S	O	N	D	J	F	M	S	O	N	D	J	F	M	N	D	P1	P2	P3	P4	P5	P6	P7	History
1	95008	1	1			1	1	1	1	1		1						1	1	1	1	1			1111100
2	95011	1				1		1		1		1	1	1	1	1	1	1	1	1	1	1	1	1	1111111
3	95025		1			1		1	1									1	1	1	1				1111000
4	95029		1			1		1					1	1	1		1	1	1	1		1	1	1	1110111
5	95032		1	1		1	1	1	1			1	1	1				1	1	1	1	1	1		1111110
6	95033		1			1						1	1	1				1	1			1	1		1100110
7	95034		1	1		1	1		1									1	1	1	1				1111000
8	95035		1			1			1		1		1	1	1			1	1		1	1	1		1101110
9	95036			1			1								1			1		1			1		1010010
10	95038			1			1	1					1	1	1			1		1		1	1		1010110
11	95069						1				1									1	1				0011000
12	95097						1						1	1	1	1	1			1		1	1	1	0010111
13	95098						1		1											1	1				0011000
14	95099						1	1					1	1	1					1		1	1		0010110
15	95100						1		1				1	1	1		1			1	1	1	1	1	0011111
16	95105						1	1												1					0010000
17	95109							1	1											1	1				0011000
18	95123							1												1					0010000
19	95124							1												1					0010000
20	95125						1	1												1					0010000
21	95127						1													1					0010000
22	95128						1									1				1				1	0010001
23	95131						1						1	1		1	1			1		1	1	1	0010111
24	95132						1				1	1		1		1	1			1	1	1	1	1	0011111
25	95133						1	1				1	1	1	1		1			1		1	1	1	0010111
26	95136					1	1				1	1	1	1	1	1	1		1	1	1	1	1	1	0111111
27	95139					1			1		1	1	1	1	1				1		1	1	1		0101110
28	95140					1		1						1					1	1			1		0110010
29	95142					1	1		1					1	1	1	1		1	1	1		1	1	0111011
30	95144					1	1												1	1					0110000
31	95145																								
32	95146					1		1								1	1		1	1				1	0110001
33	95149					1	1				1	1	1	1	1				1	1	1	1	1		0111110
34	95150					1	1		1		1	1	1	1	1	1	1		1	1	1	1	1	1	0111111
35	95151					1		1					1	1	1				1	1		1	1		0110110
36	95154					1	1												1	1					0110000
37	95155					1	1				1			1			1		1	1	1		1	1	0111011
38	95157					1									1				1				1		0100010
39	95159					1	1												1	1					0110000
40	95160					1	1									1			1	1				1	0110001
41	95163					1		1			1		1	1	1	1	1		1	1	1	1	1	1	0111111
42	95164					1		1	1		1	1							1	1	1	1			0111100
43	95165					1	1		1			1							1	1	1	1			0111100
34	95150					1	1		1		1	1	1	1	1	1	1		1	1	1	1	1	1	0111111
35	95151					1		1					1	1	1				1	1		1	1		0110110
36	95154					1	1												1	1					0110000
37	95155					1	1				1			1			1		1	1	1		1	1	0111011
38	95157					1									1				1				1		0100010

#	ID	S	O	N	D	J	F	M	S	O	N	D	J	F	M	N	D	P1	P2	P3	P4	P5	P6	P7	History
39	95159					1	1												1	1					0110000
40	95160					1	1									1			1	1				1	0110001
41	95163					1		1			1		1	1	1	1	1		1	1	1	1	1	1	0111111
42	95164					1		1	1		1	1							1	1	1	1			0111100
43	95165					1	1		1			1							1	1	1	1			0111100
41	95163					1		1			1		1	1	1	1	1		1	1	1	1	1	1	0111111
42	95164					1		1	1		1	1							1	1	1	1			0111100
43	95165					1	1		1			1							1	1	1	1			0111100
44	95168					1		1	1			1	1	1		1	1		1	1	1	1	1	1	0111111
45	95169					1	1							1		1	1		1	1			1	1	0110011
46	95173				1		1						1	1	1	1	1		1	1		1	1	1	0110111
47	95174				1	1	1						1	1	1	1			1	1		1	1	1	0110111
48	95175				1	1						1		1					1			1	1		0100110
49	95177				1		1						1	1	1	1	1		1	1		1	1	1	0110111
50	95182				1	1	1	1					1	1	1				1	1		1	1		0110110
51	95184				1	1	1							1		1	1		1	1			1	1	0110011
52	95185				1	1							1	1	1	1	1		1			1	1	1	0100111
53	95188				1	1	1	1			1	1	1	1	1	1	1		1	1	1	1	1	1	0111111
54	95193			1	1	1	1	1				1		1			1	1	1	1		1	1	1	1110111
55	95194			1		1	1				1	1	1	1				1	1	1	1	1	1		1111110
56	95196			1		1						1		1	1			1	1			1	1		1100110

S: September; O: October; N: November; D: December; J: January; F: February; M: Mars

P1 = 1 if bird present between September and November

P2 = 1 if bird present between December and January

P3 = 1 if bird present between February and March

P4 = 1 if bird present between September and November

P5 = 1 if bird present between December and January

P6 = 1 if bird present between February and March

P7 = 1 if bird present between November and December

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